Figure 16

Method for Obtaining Non-Stochastically Generated Polypeptides that can induce a Broad-Spectrum Immune Response.

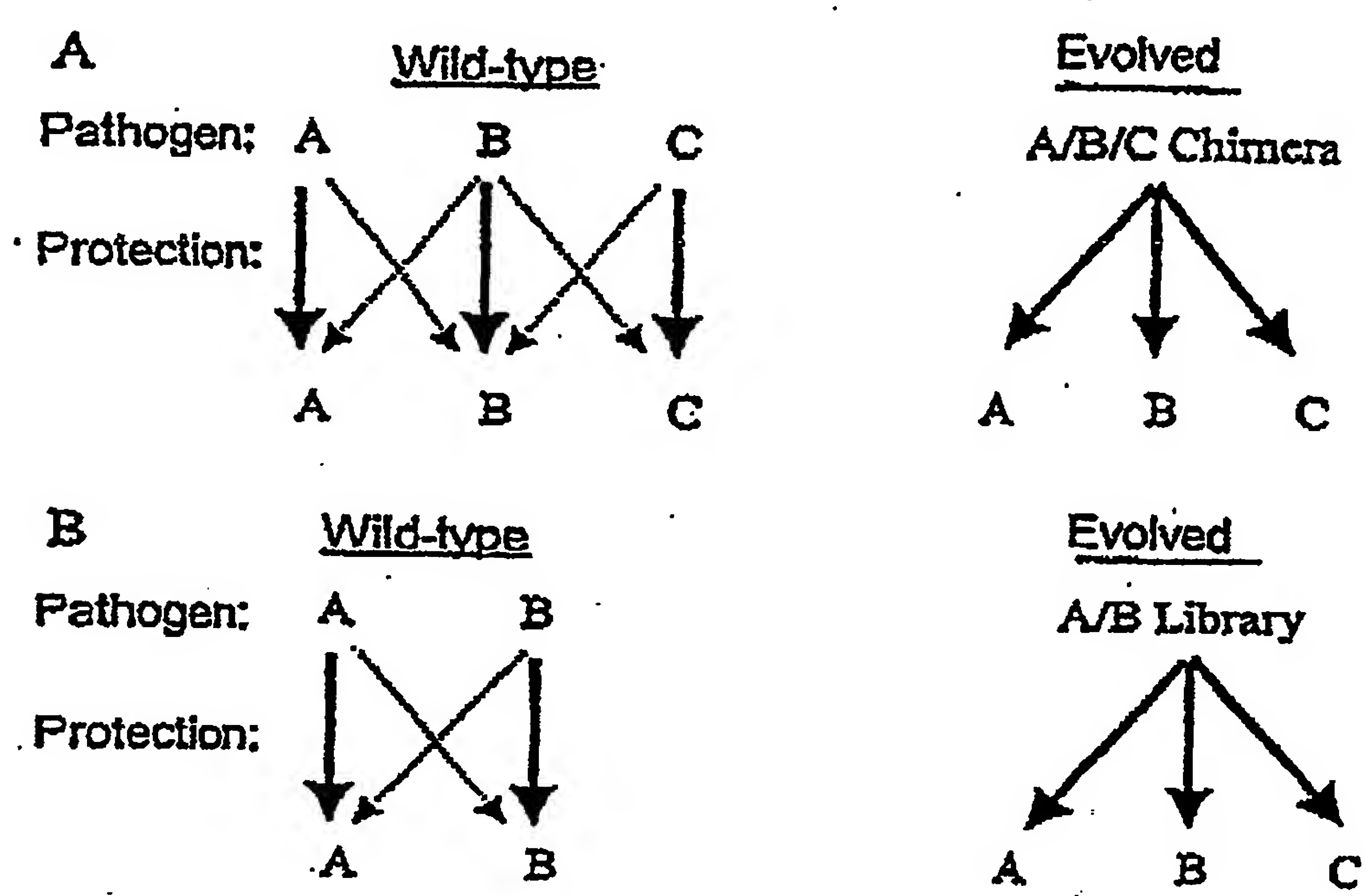


Figure 17

Possible factors for determining whether a particular polynucleotide encodes an immunogenic polypeptide having a desired property.

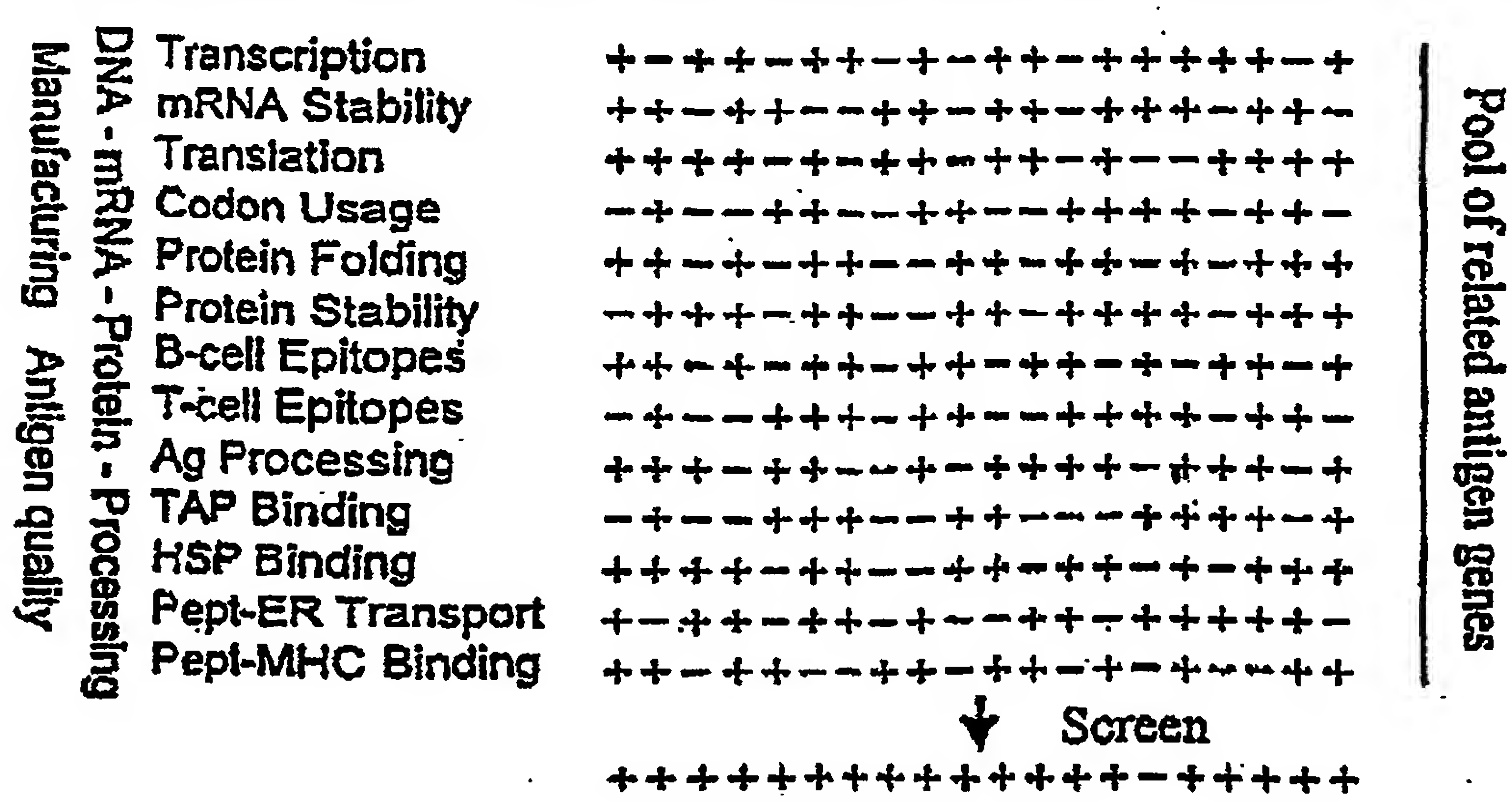


Figure 18

Screening strategy for antigen library screening.

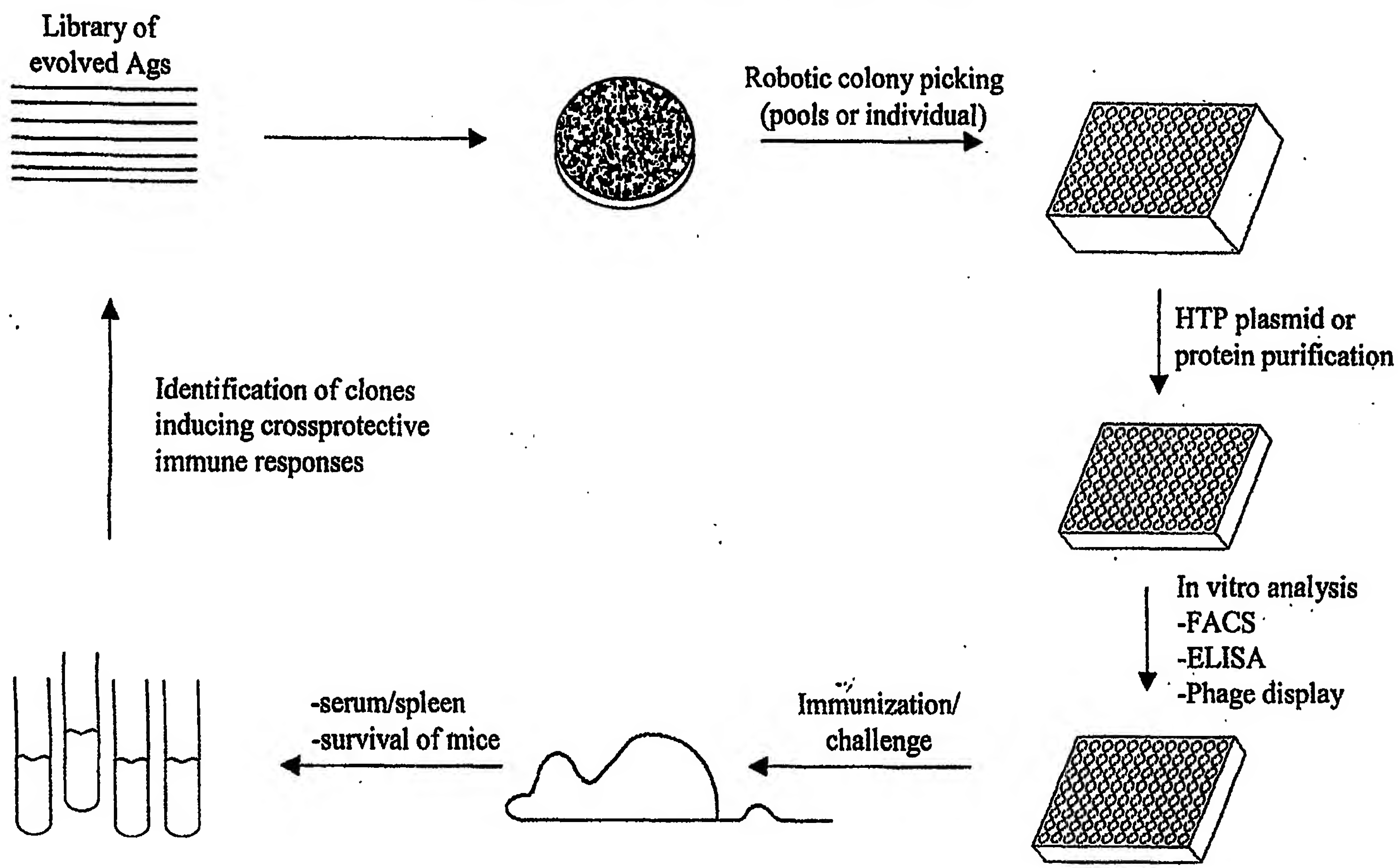


Figure 19

Strategy for pooling and deconvolution as used in antigen library screening

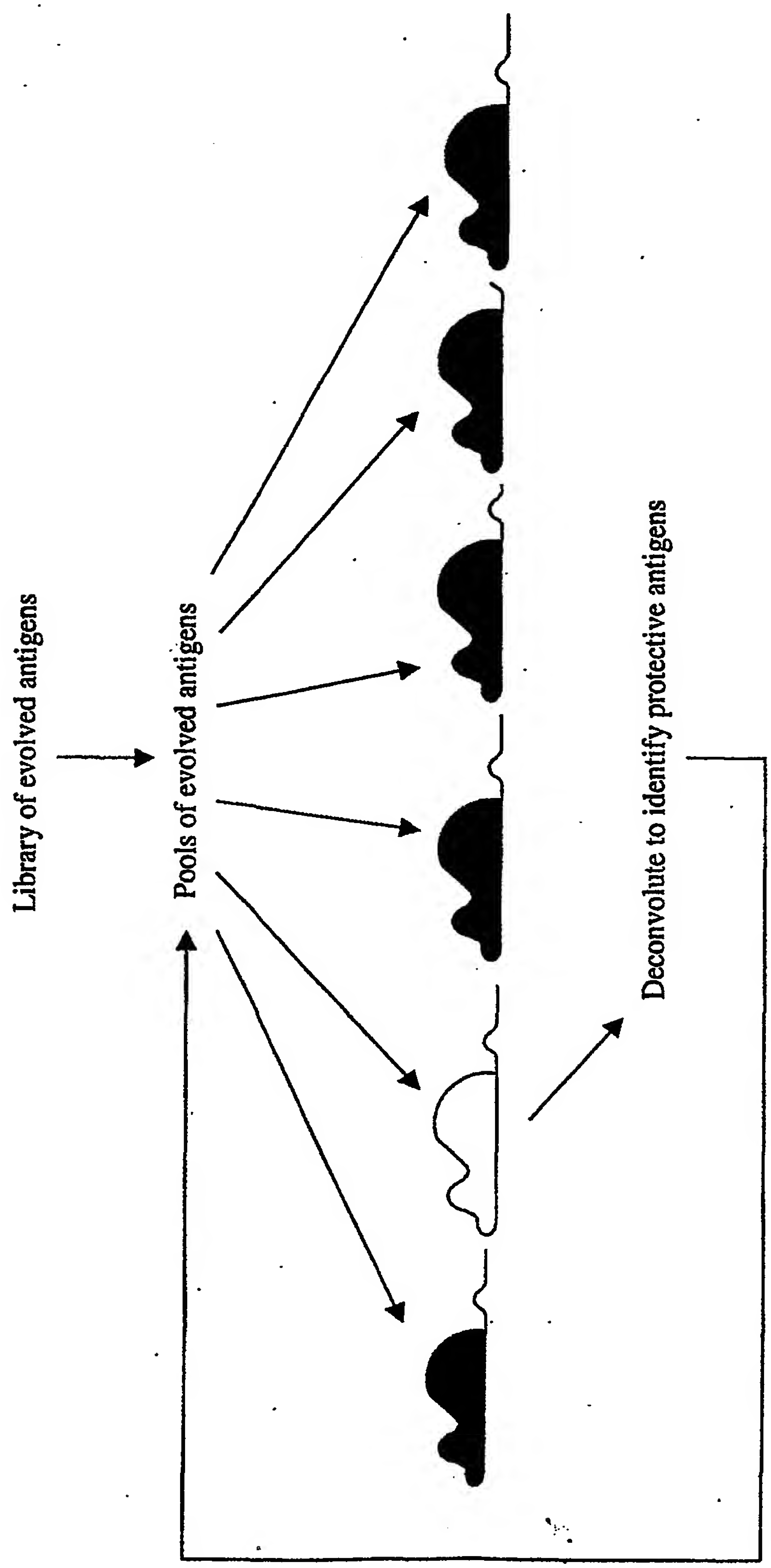
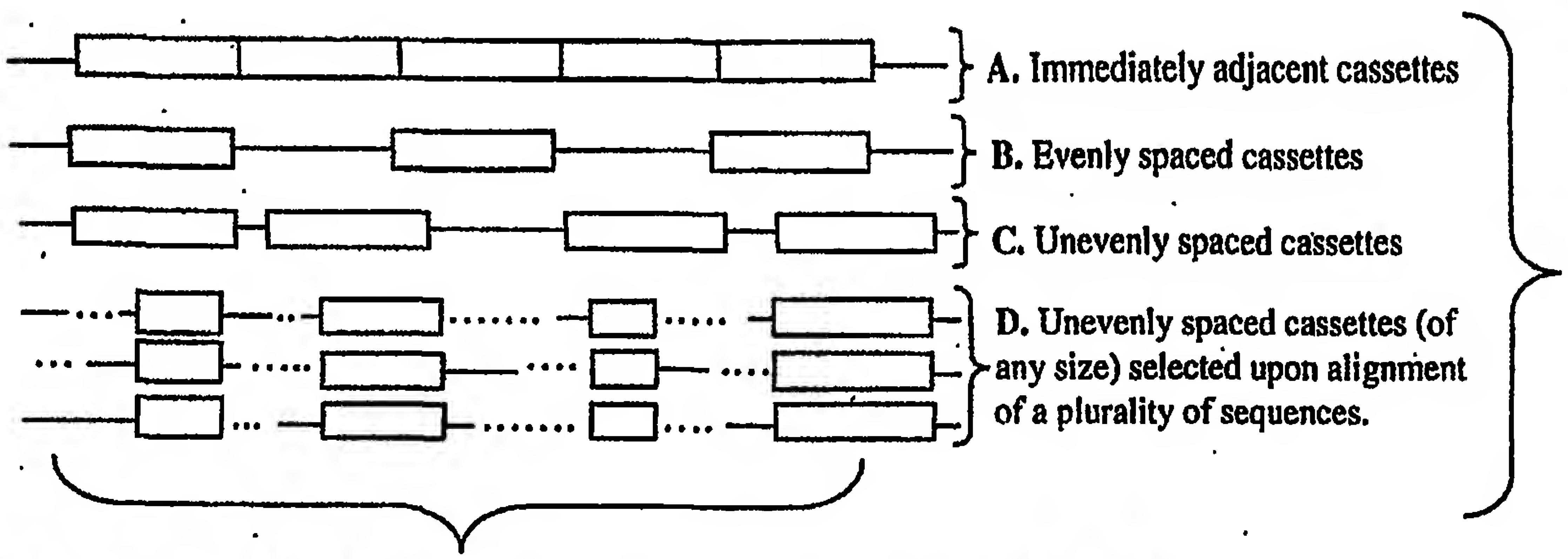


Figure 20. Preferred embodiments of site-saturation mutagenesis



I. SEQUENCES TO BE MUTAGENIZED

Nucleic acid sequences that can be mutagenized by non-stochastic site-saturation mutagenesis include any polynucleotide of 15-100,000 bases in length (e.g. any coding region & any non-coding region).

Specific examples:

- Promoter
- Enhancer
- Intron
- Whole gene
- cDNA
- Gene segment
- Operator
- Polynucleotide functional group
- Expression regulatory sequence
- Expressed sequence tag (EST)
- Open reading frame (ORF)
- Repressor/transactivator
- Origin of replication
- Gene pathway

II. MUTAGENIC CASSETTES WITHIN SEQUENCE TO BE MUTAGENIZED ($\boxed{}$)

Mutagenic cassettes that can be mutagenized by non-stochastic site-saturation mutagenesis include any polynucleotide cassette of 1-500 bases in length. Site-saturation mutagenesis is servicable for mutagenizing a complete set of cassettes contained within a polynucleotide sequence to be mutagenized. As shown, cassettes can be spaced along each polynucleotide differently (i.e. immediately adjacent, evenly spaced, or unevenly spaced) and of any size. In a preferred but non-limiting exemplification a set of mutagenic cassettes is a set of contiguous codons within a sequence of defined length. Alternatively, in another preferred but non-limiting example, a set of mutagenic cassettes is a set of nucleotide cassettes that are not shared by aligned related polynucleotides.

III. TYPES OF MUTATIONS THAT CAN BE INTRODUCED INTO MUTAGENIC CASSETTES

The type of mutations to be introduced in a set of mutagenic cassettes can be of the same type or of different types within each round of polynucleotide site-saturation mutagenesis. Each mutagenic cassette (within the nucleic acid sequence to be mutagenized) preferably is usually mutagenized by the use of a corresponding oligo (including by a degenerate oligo). Examples of degenerate mutations provided by this invention include:

- Codons for all 20 amino acids (e.g. N,N,N or N,N,G/T or N,N,G/C)
- All degenerate codons that do not change the amino acid sequence of the parental template (i.e. codons for the same amino acid that is present in the parental template)
- Codons (all or selected) for amino acids within the same grouping according to the selected amino acid grouping scheme*.
- Codons for at least 1 amino acid in each amino acids group*.

*Exemplary amino acid grouping schemes (notes, some groups overlap each other):

- Aromatic (Phe, Trp, Tyr)
- Aliphatic (Gly, Ala, Val, Leu, Ile)
- OH-containing (Ser, Tyr, Thr)
- Acidic (Asp, Glu, Asn, Gln)
- Basic (Lys, Arg, His)
- Sulfur-containing (Met, Cys)
- Polar (Ser, Thr, Cys, Asn, Gln, Tyr)
- Non-polar (Gly, Ala, Val, Leu, Ile, Met, Phe,Trp, Pro)

Figure 21

Schematic representation of a multimodule genetic vaccine vector (relative sizes of functional units are not drawn to scale)

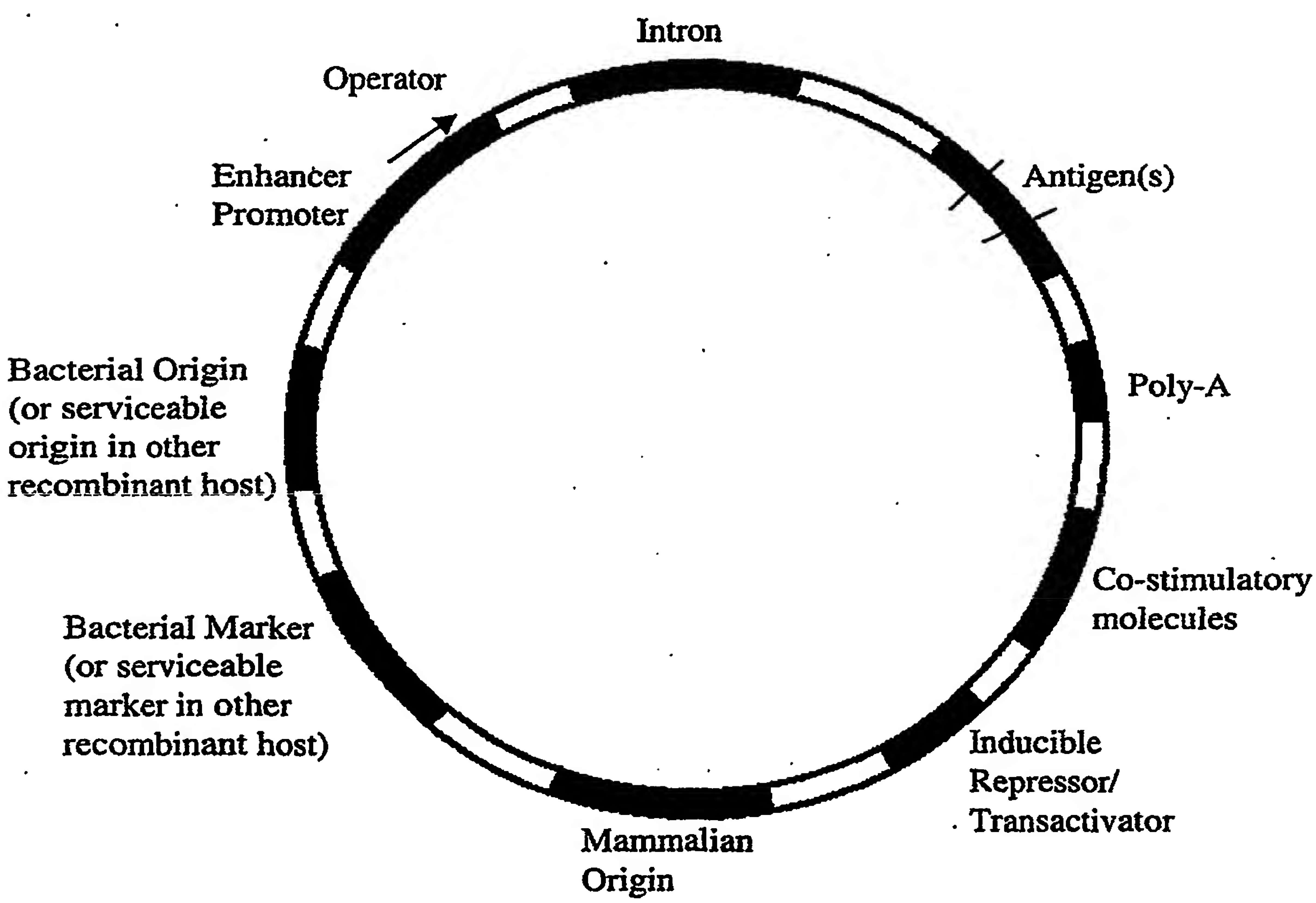


Figure 22A and 22B

Generation of vectors with multiple T cell epitopes.

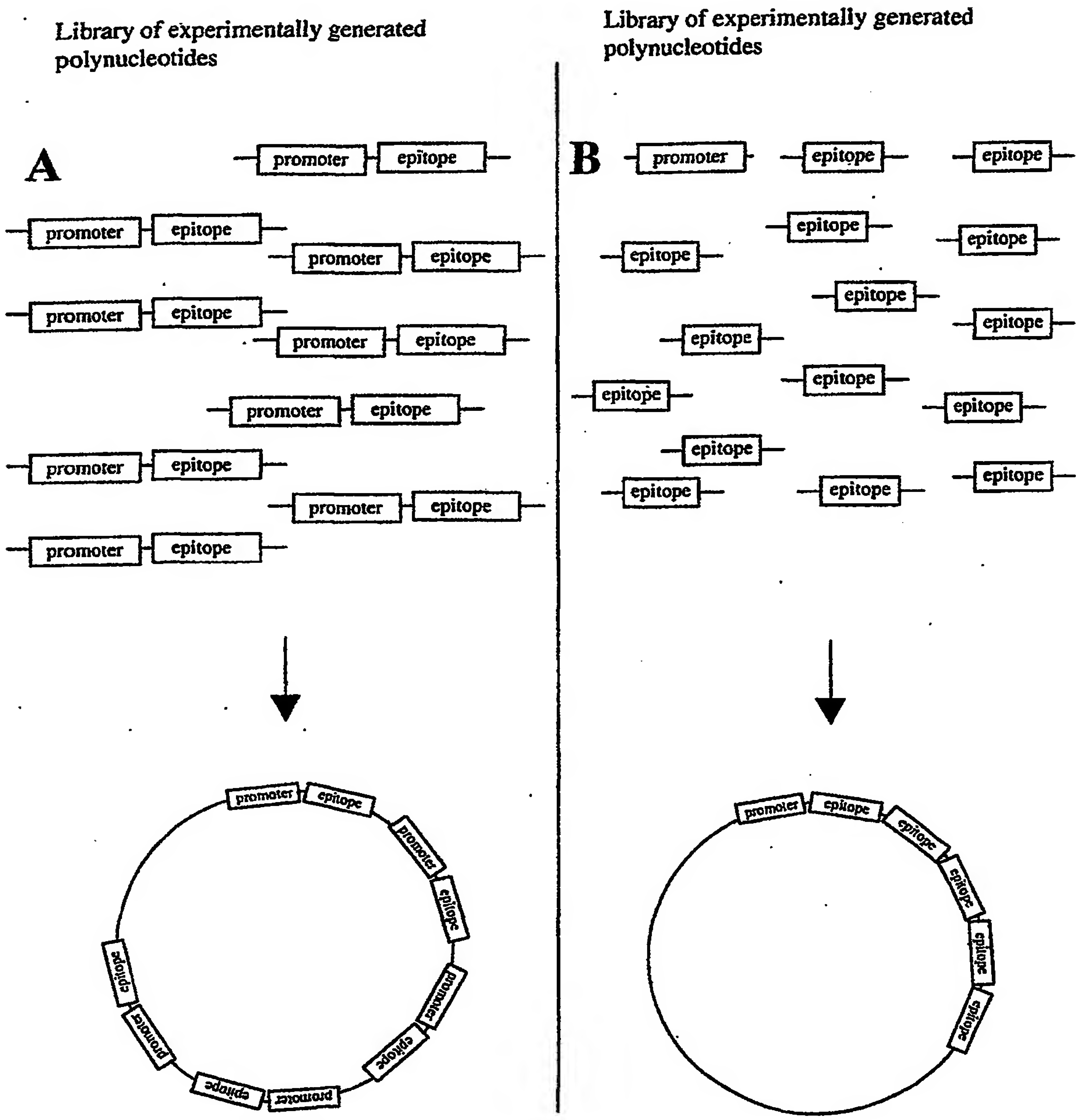


Figure 23

Generation of optimized genetic vaccines by directed evolution

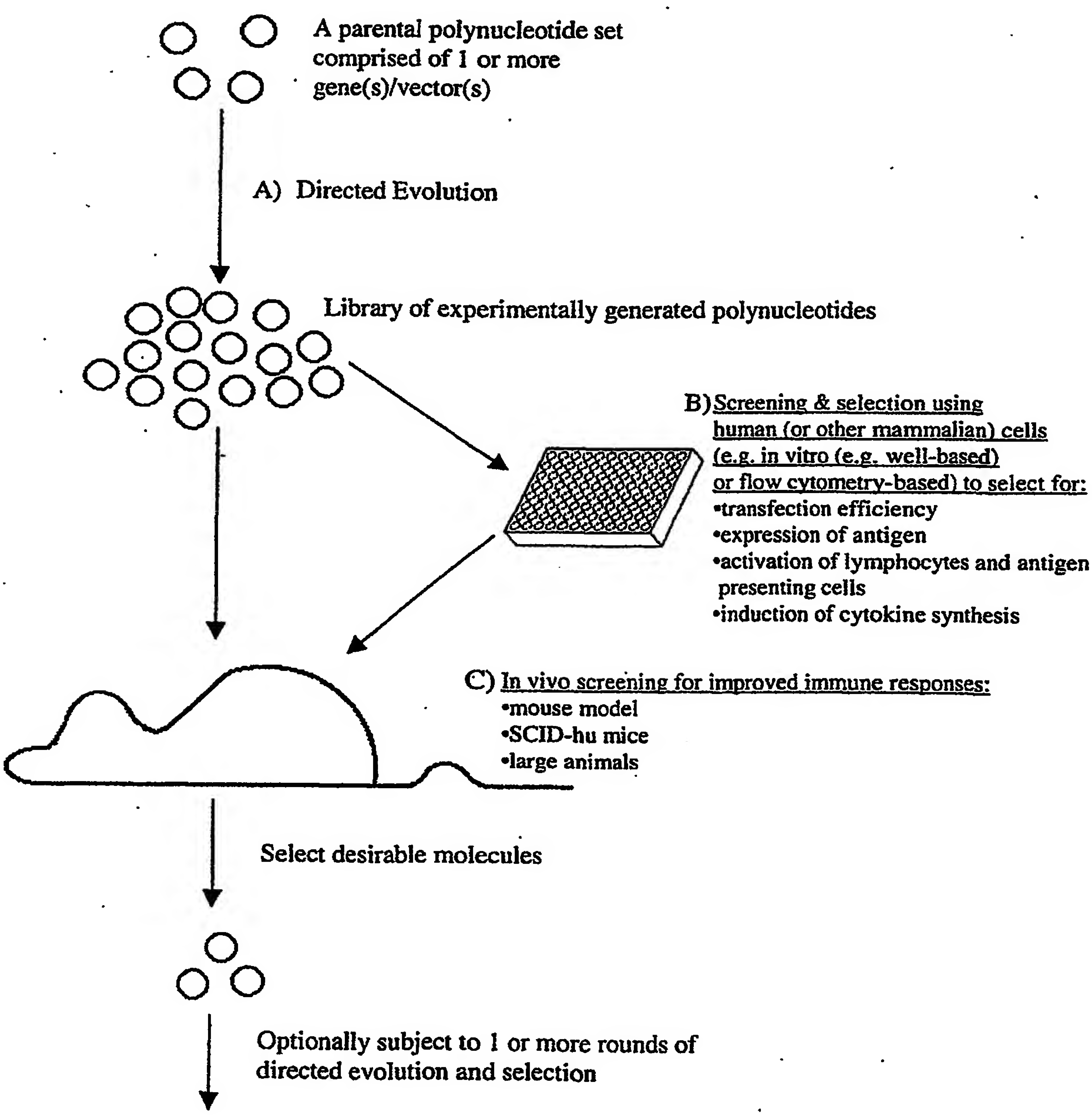


Figure 24

Recursive application of directed evolution and selection of evolved promoter sequences as an example of flow cytometry-based screening methods.

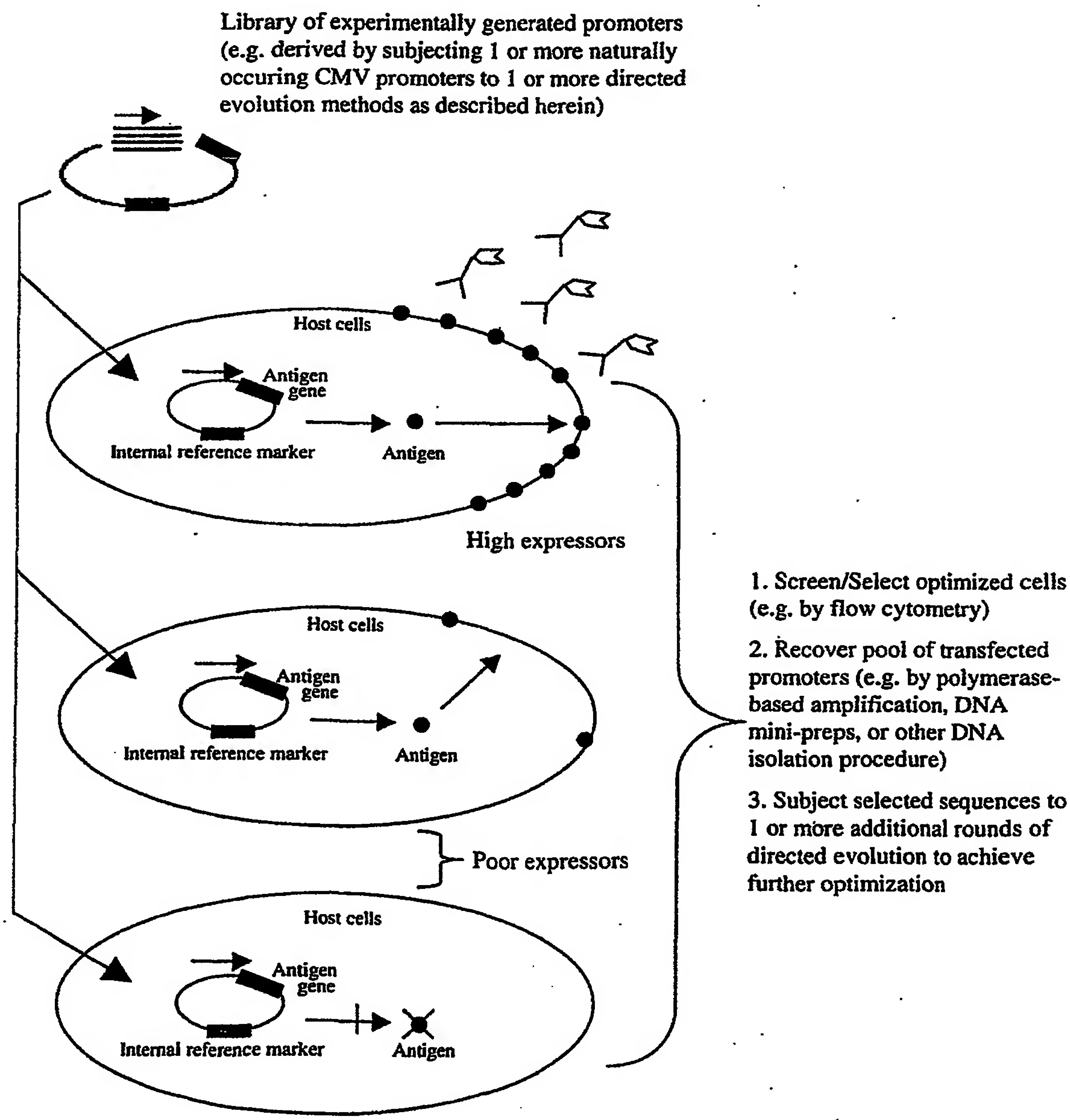


Figure 25

An apparatus for microinjections of skin and muscle.

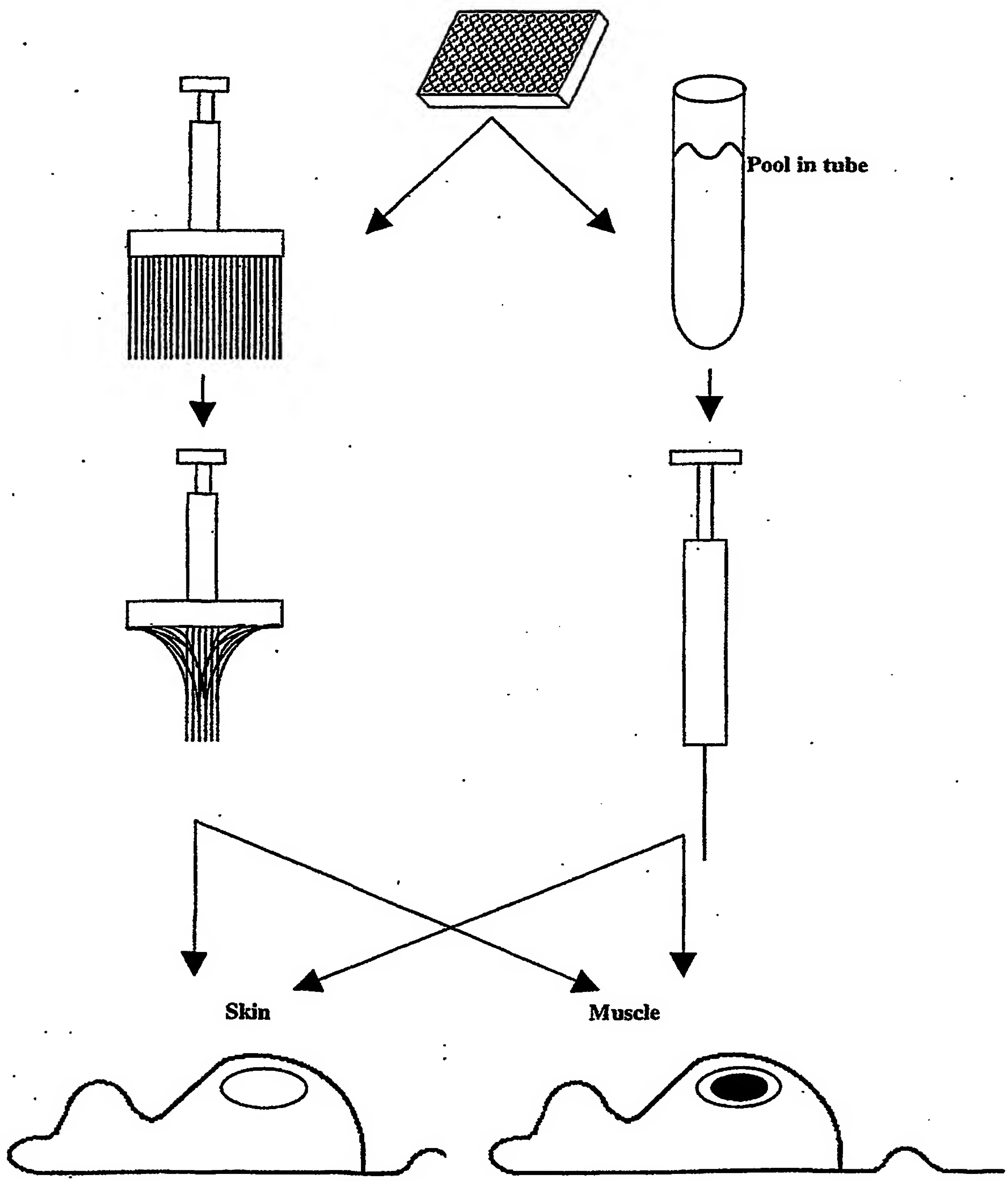


Figure 26 Panel A

Non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis.

Shown below is a non-limiting example of a permutation of the directed evolution methods described herein

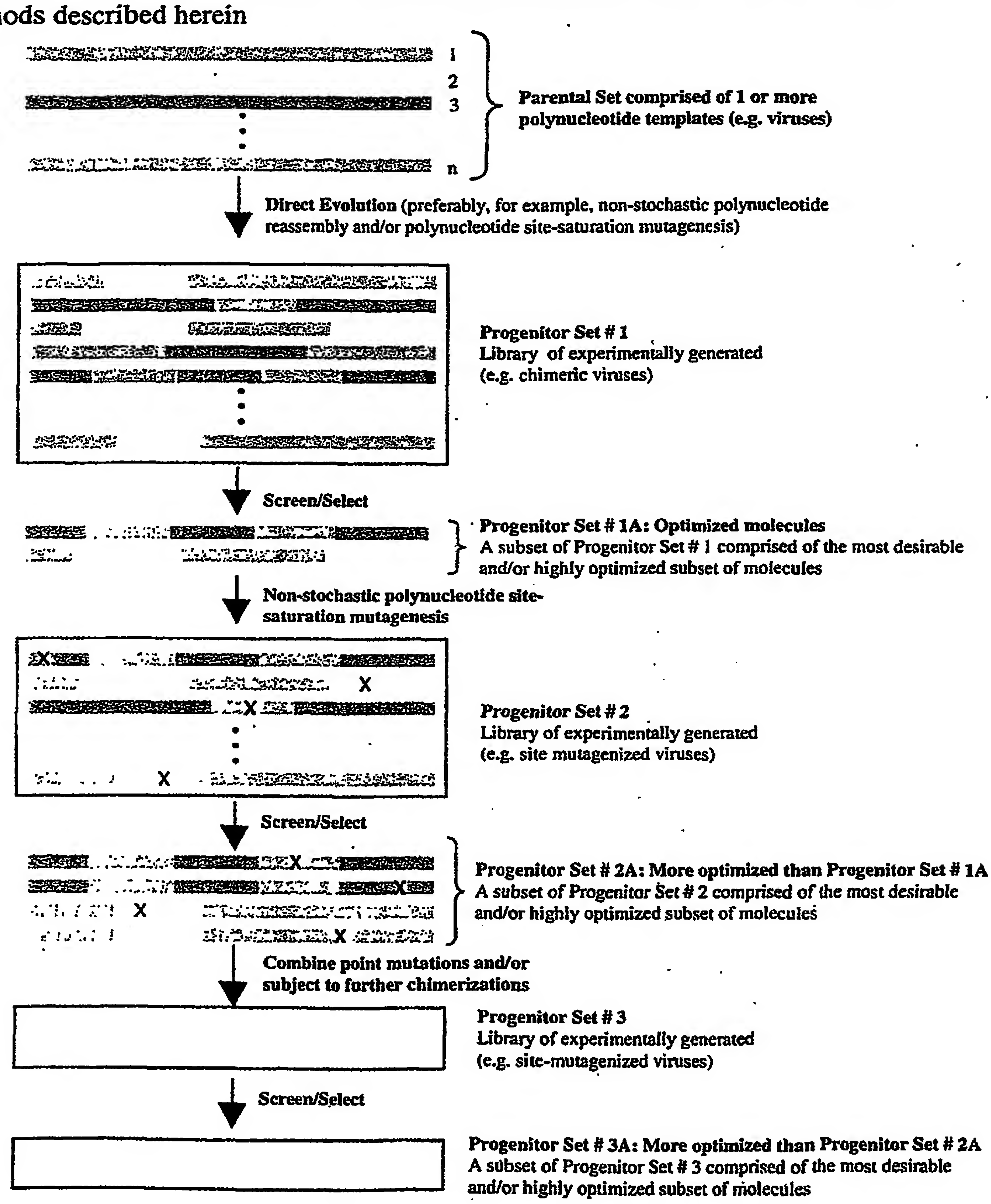


Figure 26 (continued) Panel B

Screening of experimentally generated molecules produced by non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis

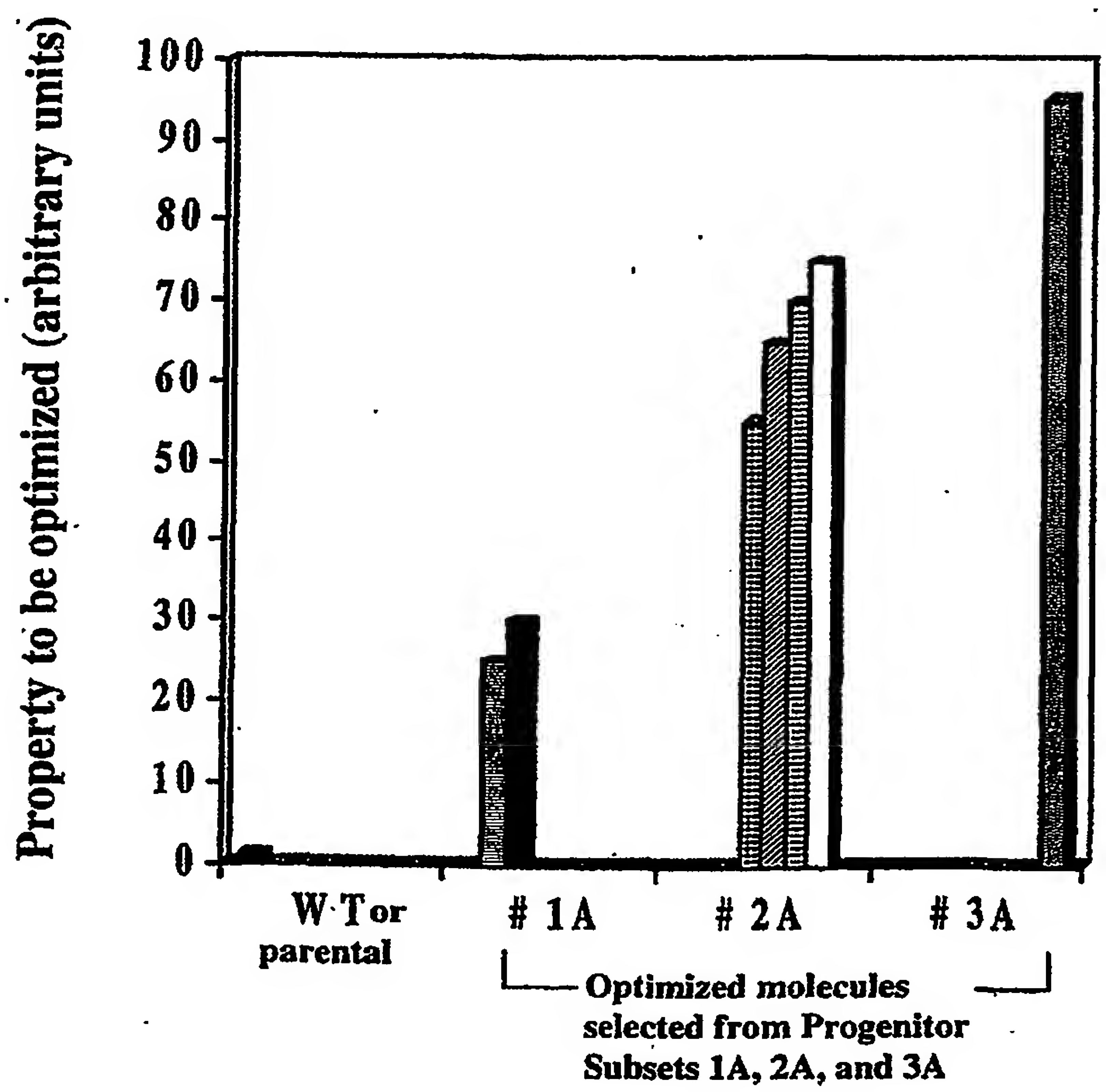


Figure 27

Vector for promoter evolution

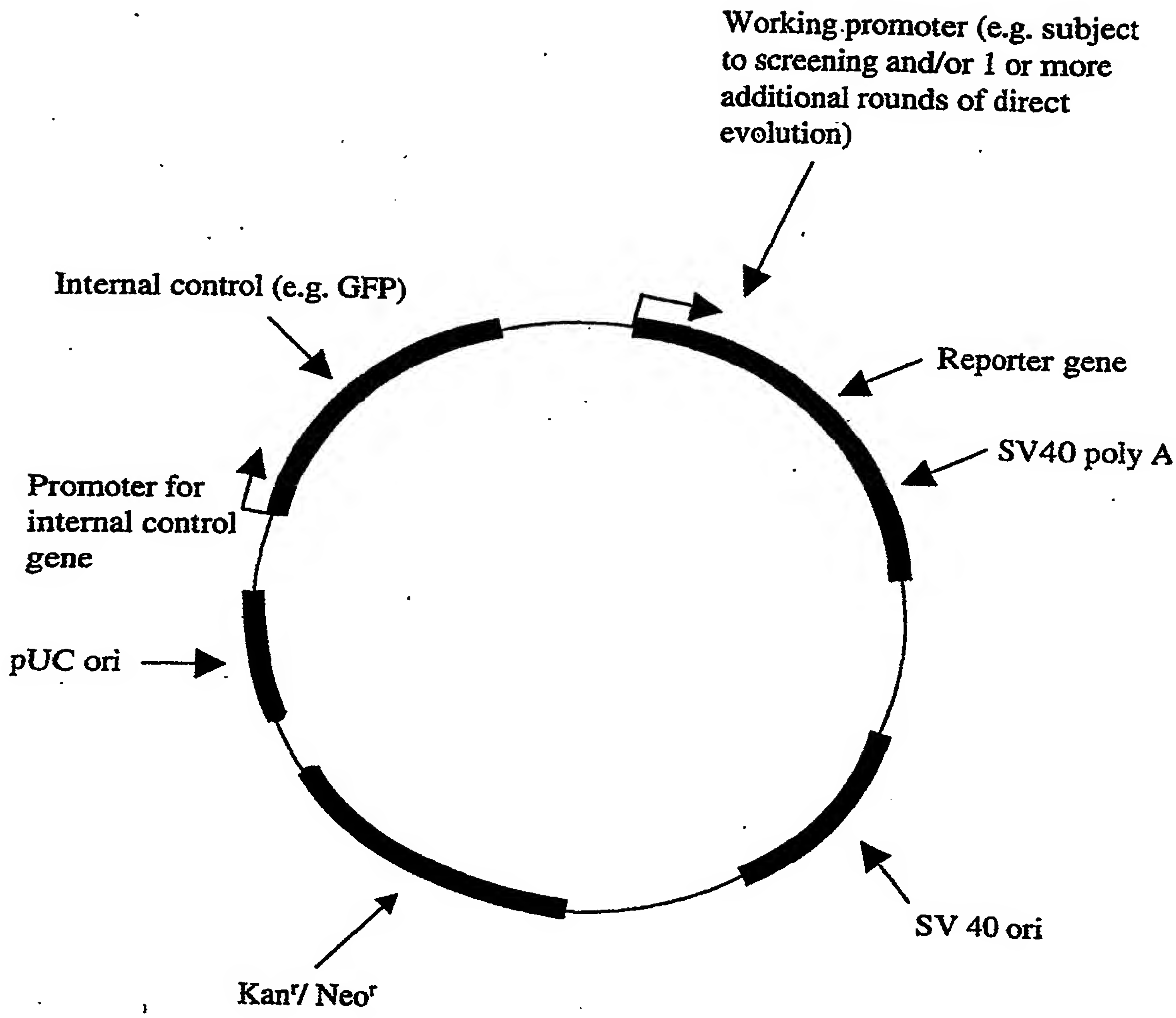


Figure 28

Iterative evolution of inducible promoters using directed evolution and flow cytometry-based selection.

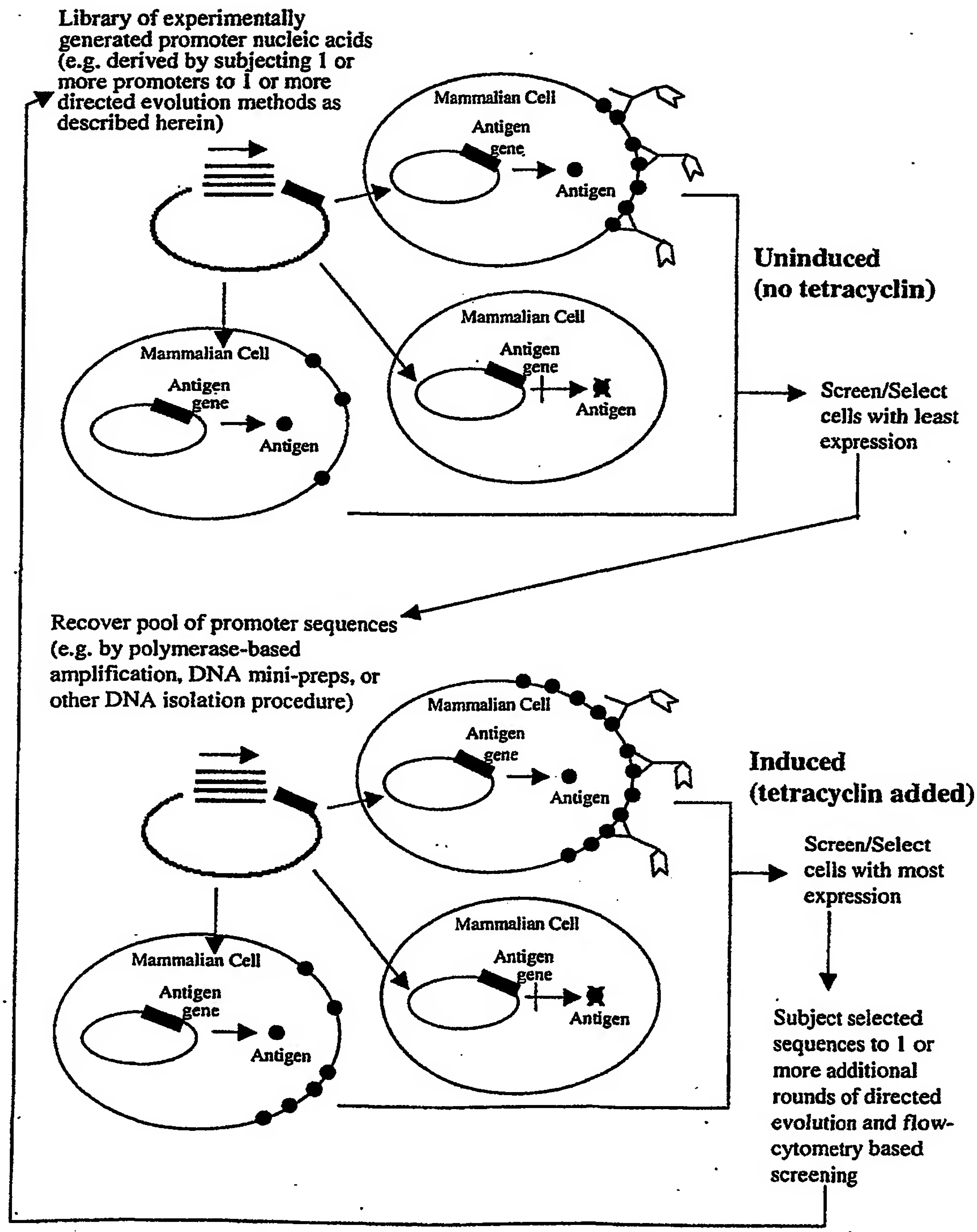


Figure 29

The present invention provides that a genetic vaccine can be subjected to directed evolution in order to achieve improved effectiveness upon administration by oral, intravenous, intramuscular, intradermal, anal, vaginal, or topical delivery methods.

The figure below shows an example of the directed evolution of a genetic vaccine, comprised of an M13 phage-based vaccine, to achieve optimization for oral delivery.

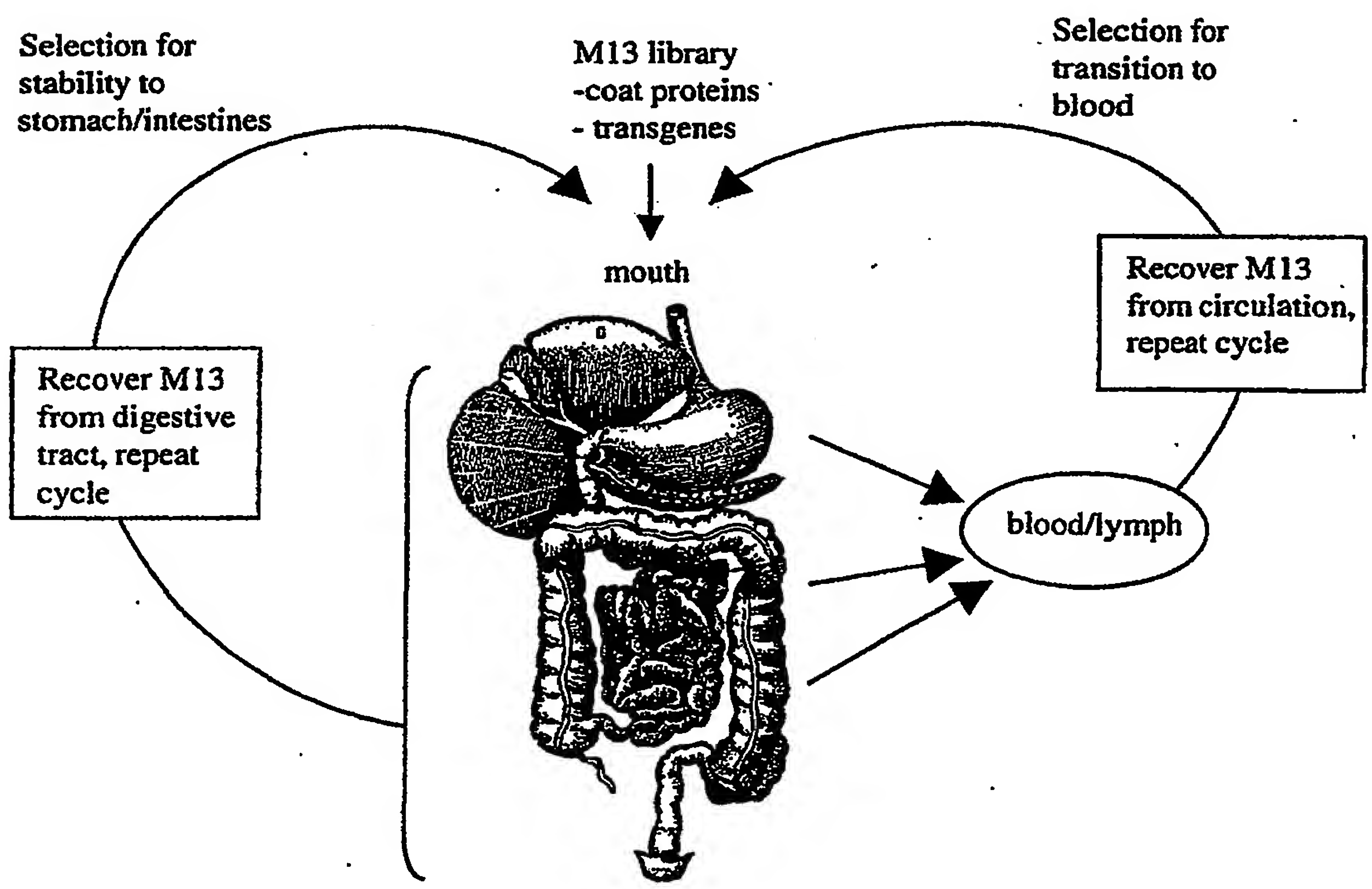


Figure 30

An alignment of the nucleotide sequences of two human CMV strains and one monkey strain.

```
                               1                                                50
AF026939 CMV         (1) -----GTGGAGAGGTG-------TTGAGCATTT-GCTTGGAATCAGTAAG
AF047524 hum UL104   (1) --GTAGTGAAATCCGTATGGACCTCCAGCACGCCGGCGGATGATCAGGGCC
AF078102 Rhesus      (1) ATGGATTTAAA-GTGGCCCGA---TTGAGGTTGTGCCCTGAACACTTTGTGA

                               51                                              100
AF026939 CMV        (38) CTAAAAAGAAAATCAACCGGGAGCCGCAGCTTTTCAGAACTGCAGGGAAA-
AF047524 hum UL104  (49) TCGATTTGGAAATGGGG--CGACAG--GCATCTGG-GCCGCGCCCCTCCTC
AF078102 Rhesus     (47) GTGTTTTGGAAGGTAGATATGACAGT-GCAGTCCAGAAGTGCCGCTGAA-

                               101                                             150
AF026939 CMV        (87) --GAGGCATCATGAGTGAGGTGACGAAGAATTGCCTGGAGAAAATGCT--
AF047524 hum UL104  (94) GTGTGCCGTGATCAGGCGGCGGGGCCG--GACCTTTGAAGCGTTCCTGG
AF078102 Rhesus     (95) GCGTGGGGTACATATCTGAAGCAGCGAGATGAGGGTCCC-GAGGAGGTGA

                               151                                             200
AF026939 CMV       (133) CGGAGAGGGGAAATGCGATTTAGAG---CTGGAACTTATTCAAGGAAGACA
AF047524 hum UL104 (142) GGCGGCGGG-GGAGGCAGTTCGGGTTTCTGGCAGTGCGGGCGCCGCTTCG
AF078102 Rhesus    (144) TAGGGTGGGAGTACGGCGTCTTGAGTTTGCGTAATGT-CGTGTTCCAACG

                               201                                             250
AF026939 CMV       (180) GTGTGTCA--AGGGATGTAGAAGATAGAGTGTG--T---AACCAGATTGA
AF047524 hum UL104 (191) CGG-GTCATTTGGCGTCGGCGCGCCTTCTCGCGGCTGCAAATCAGCTCCA
AF078102 Rhesus    (193) TTGTGTGACTATGTTAGAGGGTGTTTATCTGCGAC----AATACGATCAA

                               251                                             300
AF026939 CMV       (223) A----TTTTTAAACACTGAGTTGAAAGCTACAATGTAGAACTTGTTGGCC
AF047524 hum UL104 (240) G-GTATCGGCAAAACTTGCTGTGGTCGTAGGCGGCGGCCACGATCTCGCC
AF078102 Rhesus    (239) GCGTGTTTAGGAGAATATATTAAAAAGAATTGTGGTACTATCATTCCGTT

                               301                                             350
AF026939 CMV       (269) TACATAAAGACGTAGATGGTAAGAACGAGGCAGGCGTGGAAT-GCTTAC
AF047524 hum UL104 (289) GAAGGAGAGCTGCAGGTAGGCCTCGGGTACGG-GGTCCAGCGT-GCCCAG
AF078102 Rhesus    (289) GCGCAACCCGTGTAGCT-GGTTTGTCGTCGTTCGCTGTGGTATAGATATT

                               351                                             400
AF026939 CMV       (318) GGGAAGCTGAAGAGTTA----ATGCAGCAAGAAGATGCTGACCAAGCAGA
AF047524 hum UL104 (337) CGGCAGGATGTGACACA---GATAGGGGAGGGTCACGGGCTCTACCGTGT
AF078102 Rhesus    (338) GCCGAGGTAGAAACCCGTTCGTTGGACGAGTATCTTT-GCTGCAAAGAGA

                               401                                             450
AF026939 CMV       (364) AATCAGA--AGTGTA--GTGACGTTGGGGAAACT-ACGGCTGG-GTCTACT
AF047524 hum UL104 (384) AATTGGAGTAGACGATGGCCTGTTCGGCCCCGTGATGCGTGA--CGAGAC
AF078102 Rhesus    (387) AATTGGA--AGCACTGGCAATATTGGGGGTGCAGTTGCGTGATGGCAGTG

                               451                                             500
AF026939 CMV       (408) ATGAGTTGGGCA-----GACTCTCAGATGCTCAGATTT---ATGTAGATAA
AF047524 hum UL104 (432) GCGGCAGGCGAA---AGGTGGGGAATAGTCGTTTTCCCACAGCTGCG-T
AF078102 Rhesus    (435) ATGGCAAAAAAATCCAGGAGACTACGTGTGTGGTTCTGCTGGGAAGCTAT

                               501                                             550
AF026939 CMV       (451) GGTGAAACAAAGCTGCAAGAAATTTTCAAATCGATAGAGTATTGAGTATT
AF047524 hum UL104 (478) GAGGAAGCGTTGCAGCGACTGGGTGCGGGCACGAACTGC-GAGAAGAAG
AF078102 Rhesus    (485) GGATTTGTGTACGTGTATGAGTGGGACAGTGACGGACTCT-TTCAGATAG
```

Figure 30 continued

```
                                551                                              600
      AF026939 CMV      (501)   CTGAACTTGACTGTGAGGAAGGGTGGACACAACTGAAGTGTGGAAGAAAT
 AF047524 hum UL104     (527)   GAG--TTGGCGAGCAGG--CGGTTGTCGTGCAGCGCC-AACGGACG-GAA
    AF078102 Rhesus     (534)   GTA--GCTCAGTCAAAG--AACTCGCGCACCAGGGTT-TACTGACATGTG

                                601                                              650
      AF026939 CMV      (551)   GAAAGGGCGAAGGTGTGTTTTGAGAAGGGTGT---GGAAGAA-AAGCCCA
 AF047524 hum UL104     (571)   AGGGGCCGCGTCGCGCGGCTTCCGCACGGCCT---GCAACAC-GGGG--A
    AF078102 Rhesus     (579)   AATCGGTATATGGGCATCG--GGAAACGCCCTTCTCCACCAGCGAGCCTC

                                651                                              700
      AF026939 CMV      (597)   ACAACGCAGAATTCTCC-TCTGGACTGGCAATTGCGATGTACCATCTGGA
 AF047524 hum UL104     (615)   GGTG----GTAGAG----TTCGGCGTCGCGCGCGCCCAGGCTCATGGAGT
    AF078102 Rhesus     (627)   GTTTTCAAGTCGAGAAATTGTTGTGTGTGGATCCGACAGACGCACGTGGT

                                701                                              750
      AF026939 CMV      (646)   T-AATGACGCAGAGAAACAGTTCTCTACTGATGTTTTGAAG------CAG
 AF047524 hum UL104     (657)   GCTCGGGGGGCGAGGCGTAGCGGGTGA-GCAGGTCGGGCAGTTCGCGCAC
    AF078102 Rhesus     (677)   G-TAGGTAAGACCGCAGAAGAGTTTCATGGCGTTAACGTAGTA-GTGAAA

                                751                                              800
      AF026939 CMV      (689)   GCGATTGAGCTGAGTGCTGATAACGAAT---ACCTGAAGGTTGTCTTGGG
 AF047524 hum UL104     (706)   GGGATTCTCGCAGGTGTGGTTGAGGGTGCGGAGGTCCTGGATCTCGTCTA
    AF078102 Rhesus     (725)   ACGCCTGGGGGATCGGAGGCCGATGCCTTGCTGCTTCTGGGTACTGTGGA

                                801                                              850
      AF026939 CMV      (736)   CCTGAAACTGCAGAAGATGAATAAAGA--AGCTGAAGGAGAGCAGTTTGT
 AF047524 hum UL104     (756)   CGTGCGACTGGATCTGCTCGTGCAGGG--ACTTGAT---GACCTGCTTCT
    AF078102 Rhesus     (775)   GAAGGTAGGTCAAATGTATCCGTTTGCTAAAATGA----GAGCTGATAAC

                                851                                              900
      AF026939 CMV      (784)   TGAAGAAGCGTTGGAAAAGTCTCCTTGCCAAACAGATGTCCTCCGCAGTG
 AF047524 hum UL104     (801)   TAAACAGGTGGCGGGATG-TCCGGCTCGGGGCCGGCGGGCCG--GGTG-G
    AF078102 Rhesus     (821)   TTTAGTAATCTCATAAA-GTATATTAAT-CAACGCATGT-----GTTG-C

                                901                                              950
      AF026939 CMV      (834)   GAGGCAAATTTTAGAGAAGAAAAGGTGACCTAGACAAAGCTATTGAACTG
 AF047524 hum UL104     (847)   GGCGCGGCAGCAGGCCGACGTGGCCCGGGGTCCTGCCA-GCACGGCACCG
    AF078102 Rhesus     (863)   CGATGGTACGTACTTGGTGTTACTGGGCGTTACGGTAA-TTTTGGAATTT

                                951                                             1000
      AF026939 CMV      (884)   TTTCAACGGGTGTTGGAATCGACACGAAACAATGGCTACCTCTATCACCA
 AF047524 hum UL104     (896)   CCGGGCCGC----ACCACGGCGGGTGCACGCGGACCAGGCGCGGGTAGCA
    AF078102 Rhesus     (912)   TGCTGACGTGTGGAATAATTGTGTTGGACGAGTGCGGCGTGTGT-TATGT

                                1001                                            1050
      AF026939 CMV      (934)   GATTGGGTGCTGGTACAAGGCAAAAGTAAGACAAATGCAGAATACAGGAG
 AF047524 hum UL104     (942)   GACGGTTTTGGTCCACCAGCGAAGGGGTCAAGTCCTGCAGG------AAG
    AF078102 Rhesus     (961)   GCTGAGAATCGACGAA-AGCGACGTGTTTC-GTCTAGCCGAT-----AAC

                                1051                                            1100
      AF026939 CMV      (984)   AATCTGAAGCTAGTGGAAATAAAGAGATG--ATTGAAGCACTAAAGCAAT
 AF047524 hum UL104     (986)   GACTCGACGCTGTCCTCGATGCCGATGCGCGATTTGCTGTCCGAGACGTT
    AF078102 Rhesus    (1004)   ATCGAAATGTTGTTCA-GATGCGGCTTTC----TTAAGCTCCGTGGCATT

                                1101                                            1150
      AF026939 CMV     (1032)   ATGGTATGGACTATTCGAATAAAGCTCTTGAGAAGGGACTGAATCCTCTG
 AF047524 hum UL104    (1036)   AAGCAAAAACTTCATAATGGACTTTT-TGGCGTCGCT-GCCCCGGTCGTG
    AF078102 Rhesus    (1049)   ACGCGAT---TTGACCGCGGATTGCG-TGGCGAGGCTCGTCTGGAGAGTG
```

Figure 30 continued

```
                          1151                                              1200
   AF026939 CMV   (1082)  AATGGATACTCCGATCTGGCTGAG-TTGCTGGAGACGGAATGTTATCAGA
AF047524 hum UL104 (1084)  C-TGG-TCCATG-ATGTGCACCAGCTTGTTGCAGTTGAGCTCGTGGCGGC
 AF078102 Rhesus  (1095)  AGTGG-TAGTGGTATCAGGTGCCCGTAAGCAAGATCT-CTCTTGGTGCT

                          1201                                              1250
   AF026939 CMV   (1131)  GACGATTGAATTAAGGAAGTGCGTGAT--GCTGAAAAGGAAGAATG----G
AF047524 hum UL104 (1131)  TGGCGGTGACCGAG---TTTGAGAGGAAAGGTATTGAGGAACTGGGAGATC
 AF078102 Rhesus  (1143)  GTATGGTGAACAGC---AGTTAGCGCC--------GAGGAGGCTC-------

                          1251                                              1300
   AF026939 CMV   (1175)  CATCAGCG-GTAGTGCAAGGTTGGA--GAAATATAATGGGAAGTCTGAAGA
AF047524 hum UL104 (1178)  TTATGGTGGGGGCAGAGCCGCGTGGTAGCGCAG-AATGTG-CTCGTGCAGG
 AF078102 Rhesus  (1175)  -TGTGGTG-GGTACGACTGGCTGAC-GCGCAGTAATCGGGCTCATGATAA

                          1301                                              1350
   AF026939 CMV   (1222)  CACTGGTGTGGAAGATGGTTTAGAGG-GTTTGTC-----CATAAGCAAAA
AF047524 hum UL104 (1226)  TGTGCCAC--GGGGGTGGTGAACAGCAGCTTGTTGCGCTCATAAGCCAGC
 AF078102 Rhesus  (1222)  AATAGGTA--GCAGTTGTTGTTGGGGACGTAGT---GATTTGATGCCTAC

                          1351                                              1400
   AF026939 CMV   (1266)  AATCAACTGAGAAG-GAAGAGATCAAAGACGAACGACAGAATGTATCCGA
AF047524 hum UL104 (1274)  GGTTCGGTCGGCAC-GTACAAGCGGATGTGGTTGGCGCGCAGCTGCGCCT
 AF078102 Rhesus  (1267)  CGGAGTATTGCAAGAGAATCAGAATTGGTGGTTTCCGTGGAGCAGTGTCA

                          1401                                              1450
   AF026939 CMV   (1315)  AAATCTGGTTGCACAAAATGGACCAAATTATTG-----GTATCTTCAAGGA
AF047524 hum UL104 (1323)  GCAGCCGCTCG----GAGCGCACCTTCTTGAAGACGCGTACCT--CGGGC
 AF078102 Rhesus  (1317)  C--GGTTGTAG----AAGGGGCCAAAGCGGTG---CGTGGGA--AGAAA

                          1451                                              1500
   AF026939 CMV   (1361)  TTAATTCATAAGGAGAATGGAGATGTGGTGGAAGCAGCGAAATGTTATGA
AF047524 hum UL104 (1367)  GCGTTGGCTACGCGCA---CAGCTGCCAGGGGCTCGGGCTCCTGCAGCA-
 AF078102 Rhesus  (1356)  ATGATGAGTTAACGGA---GAAAACGTGTGATGGTACTGTGATGTTTCGT

                          1501                                              1550
   AF026939 CMV   (1411)  GAAGGAAGTGGGCCGCGTGG--TAAGGGATGGCGGTTGAGGCAT--AGGC
AF047524 hum UL104 (1413)  GCAGCGCCCAGGTTAGCGTGGAGCAGGTGCTGGCGCCAGCGGGTGTGTCT-C
 AF078102 Rhesus  (1403)  GTAGACAGAGGTTTTTCTTCG--GAGAAGGGTAAAGTTGGAGGGTGAAAGC

                          1551                                              1600
   AF026939 CMV   (1457)  AGTATTTTGCTGTCAGCATGTGAGGTTGAGGATGGT-AGTGAGGAAATGG
AF047524 hum UL104 (1462)  GGTGGGT-GGTGGAC-GGGCGCGGGTACAAATTGG--GCGCGCTCGGCG
 AF078102 Rhesus  (1451)  GATGAGTACAGAGAAGAGGAGGAAGATGATGATAAGCAGTCAGAAGAAAC

                          1601                                              1650
   AF026939 CMV   (1506)  GGGAGGGG-GGAGTCAGCTGCA---GTGCCAGAGAGCTCGTCTGTAACTC
AF047524 hum UL104 (1508)  GCGTG-GG-TGGGCTTGGTGGTTCACGTGCAGCAGCGGTAG---CAGTCCC
 AF078102 Rhesus  (1501)  CGGTGAGGATGAGCCTGTTGTCATTGGTTCACAT-GAAAG---AAACGGC

                          1651                                              1700
   AF026939 CMV   (1552)  AGAGCAACTGAACTGAGACAGAGGAGGAAACAGAGC--ATCAGAAGCCT
AF047524 hum UL104 (1553)  ACCGTTAC-GCACCAATCGACGTAGAGACCATAGTCGTCGTTATGGCGT
 AF078102 Rhesus  (1547)  AGTGTTG--------ATTGTTATGATGATGAGAGTGA--GTCATCTCTGT

                          1701                                              1750
   AF026939 CMV   (1600)  GGAGTGGTGGTTGTGACGGGTAGGAGGATAGGAAGAGAG-GGGGCGCCAA
AF047524 hum UL104 (1602)  ACTGATATAAA-ATGTCGCG---GAGCGCGGGAG-G---ACGCCCGTTT
 AF078102 Rhesus  (1587)  CGTCAGATACCCATGATCG-----ACGAGCCCCAGACATTATGGCCATAA
```

Figure 31

An alignment of IL-4 nucleotide sequences from 3 species (human, primate, and canine).

```
                                        1                                                50
       AF187322 Canis IL-4      (1)     TGCATCGTTAGCGTCTCCTAGTAAACTGATTTGTCTGCTATTGTCACTGC
  NM_000589 Homo sapien IL-4    (1)     TGCATCGTTAGCTTCTCCTGATAAACTAATT-GCCTCACATTGTCACTGC
     U19838 Cercocebus IL-4     (1)     --------------------------------------------------

                                        51                                              100
       AF187322 Canis IL-4     (51)     AAATAGAGATCTATTAATGCCTCTCACCTGCCAACTGATTCCAACTCTGG
  NM_000589 Homo sapien IL-4   (50)     AAATCGACACCTATTAATGGGTGTCACCTCCCAACTGCTTCCCCCTCTGT
     U19838 Cercocebus IL-4     (1)     ----------------ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGT

                                        101                                             150
       AF187322 Canis IL-4    (101)     TCTGCTTACTAGCACTCACCAGCACCTTTGTCCACGGACATAACTTCAAT
  NM_000589 Homo sapien IL-4  (100)     TCTTCCTGCTAGCATGTGCCGGCAACTTTGTCCACGGACACAAGTGCGAT
     U19838 Cercocebus IL-4    (35)     TCTTCCTGCTAGCATGTCGCGGCAACTTTGCCCATGGACACAACTGCCAT

                                        151                                             200
       AF187322 Canis IL-4    (151)     ATTACTATTAAACACATCATCAAAATCTTGAACATCCTCACAGCGAGAAA
  NM_000589 Homo sapien IL-4  (150)     ATCACCTTACAGGAGATCATCAAAACTTTGAACAGCCTCACAGAGC-AGA
     U19838 Cercocebus IL-4    (85)     ATCGCCTTACGGGAGATCATCGAAACTCTGAACAGCCTCACAGAGC-AGA

                                        201                                             250
       AF187322 Canis IL-4    (201)     CGACTC-GTCCATGGAGCTGACTGTCAAGGACGTCTTCACTGCTCCAAAG
  NM_000589 Homo sapien IL-4  (199)     AGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCCAAG
     U19838 Cercocebus IL-4   (134)     AGACTCTGTGCACCAAGTTGACGATAACGGACATCCTTGCTGCCTCCAAG

                                        251                                             300
       AF187322 Canis IL-4    (250)     AACACAAGCGATAAGGAAATCTTCTGCAGAGCTGCTACTGTACTGCGGCA
  NM_000589 Homo sapien IL-4  (249)     AACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCA
     U19838 Cercocebus IL-4   (184)     AACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCA

                                        301                                             350
       AF187322 Canis IL-4    (300)     GATCTATACACACAA---------------CTGG----------------
  NM_000589 Homo sapien IL-4  (299)     GTTCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCAC
     U19838 Cercocebus IL-4   (234)     GTTCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGAGTGCAC

                                        351                                             400
       AF187322 Canis IL-4    (319)     ------TCCA-----ACA------------GATATCTCAGAGGACTCTAC
  NM_000589 Homo sapien IL-4  (349)     AGCAGTTCCACAGGCACAAGCAGCTGATCCGATTCCTGAAACGGCTCGAC
     U19838 Cercocebus IL-4   (284)     AGCAGTTCCACAGGCACAAGCAGCTGATCCGATTCCTGAAACGGCTCGAC

                                        401                                             450
       AF187322 Canis IL-4    (346)     AGGAACCTCAGCAGGATGGCAAACAAGA---CCTGTTCTATGAATGAAAT
  NM_000589 Homo sapien IL-4  (399)     AGGAACCTCTGGGGCCTGGCGGGCTTGAATTCCTGTCCTGTGAAGGAAGC
     U19838 Cercocebus IL-4   (334)     AGGAACCTCTGGCGCCTGGCGGGCTTGAACTCCTGTCCTGTGAAGGAAGC

                                        451                                             500
       AF187322 Canis IL-4    (393)     CAAGAAGAGTACACTGAAAGACTTCTTGGAAAGGCTAAAAGTGATCATGC
  NM_000589 Homo sapien IL-4  (449)     CAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTAAAGACGATCATGA
     U19838 Cercocebus IL-4   (384)     CAGCCAGAGTACGTTGGAAGACTTCTTGGAAAGGCTAAAGACGATCATGA

                                        501                                             550
       AF187322 Canis IL-4    (443)     AGAAGAAATACTACAGGCATTGAAGCTGAATATTTTAATTTATGAGTTTT
  NM_000589 Homo sapien IL-4  (499)     GAGAGAAATATTCAAAGTGTTCGAGCTGAATATTTTAATTTATGAGTTTT
     U19838 Cercocebus IL-4   (434)     GAGAGAAATATTCAAAGTGTTCGAGCTGAA--------------------
```

Figue 31 continued

```
                                      551                                              600
     AF187322 Canis IL-4      (493)  TACATAGCTTTATTTTAAAATATTTATA-ATTTATA------GATAATA
  NM_000589 Homo sapien IL-4  (549)  TG-ATAGCTTTATTTTTTAAGTATTTATATATTTATAACTCATCATAAAA
     U19838 Cercocebus IL-4   (464)  --------------------------------------------------

                                      601                                 637
     AF187322 Canis IL-4      (536)  TAA--TATATATAGACTCTAATATAAAAAAAAAAAAA
  NM_000589 Homo sapien IL-4  (598)  TAAAGTATATATAGAATCTAA----------------
     U19838 Cercocebus IL-4   (464)  -------------------------------------
```

Figure 32

Evolution of polypeptides by synthesizing (in vivo or in vitro) corresponding deduced polynucleotides and subjecting the deduced polynucleotides to directed evolution and expression screening subsequently expressed polypeptides.

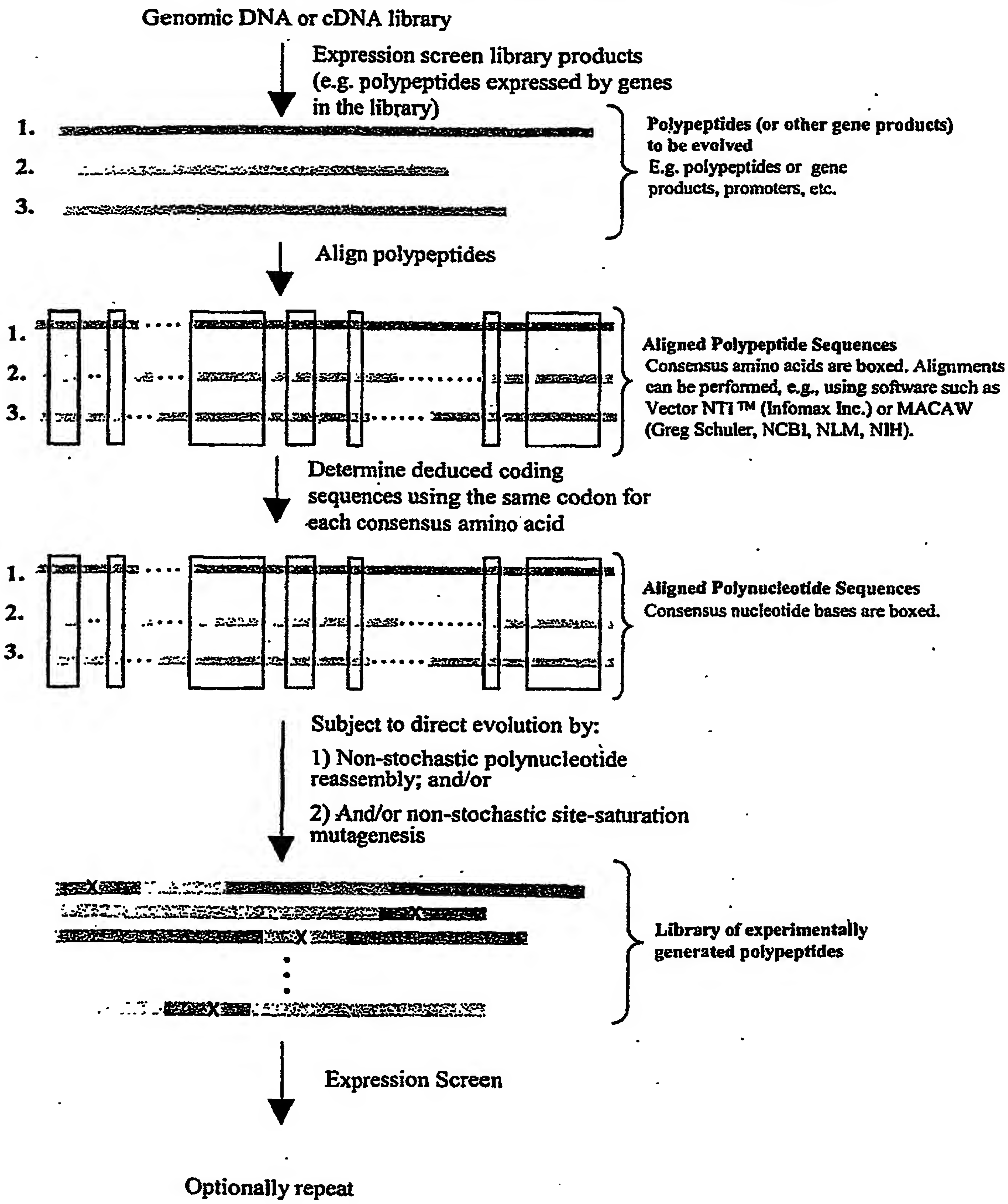


Figure 33

Directed evolution of polynucleotides (e.g. promoter sequences)

This figure shows an example of the application of non-stochastic site-saturation mutagenesis in combination with non-stochastic reassembly (e.g. oligo-directed CpG deletion(s) and/or addition(s))

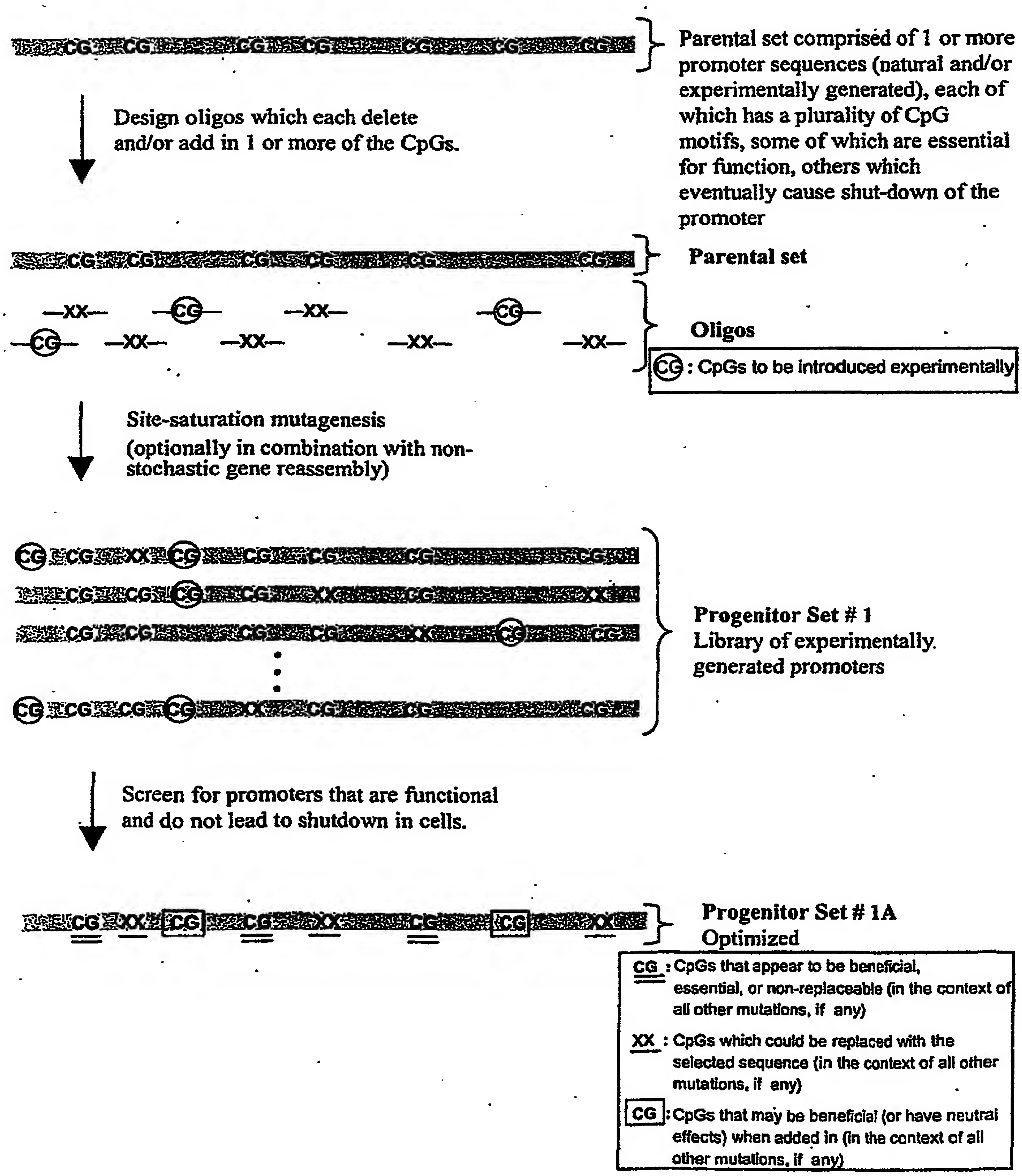


Figure 34

An example of a CTIS obtained from HbsAg polypeptide (PreS2 plus S regions).

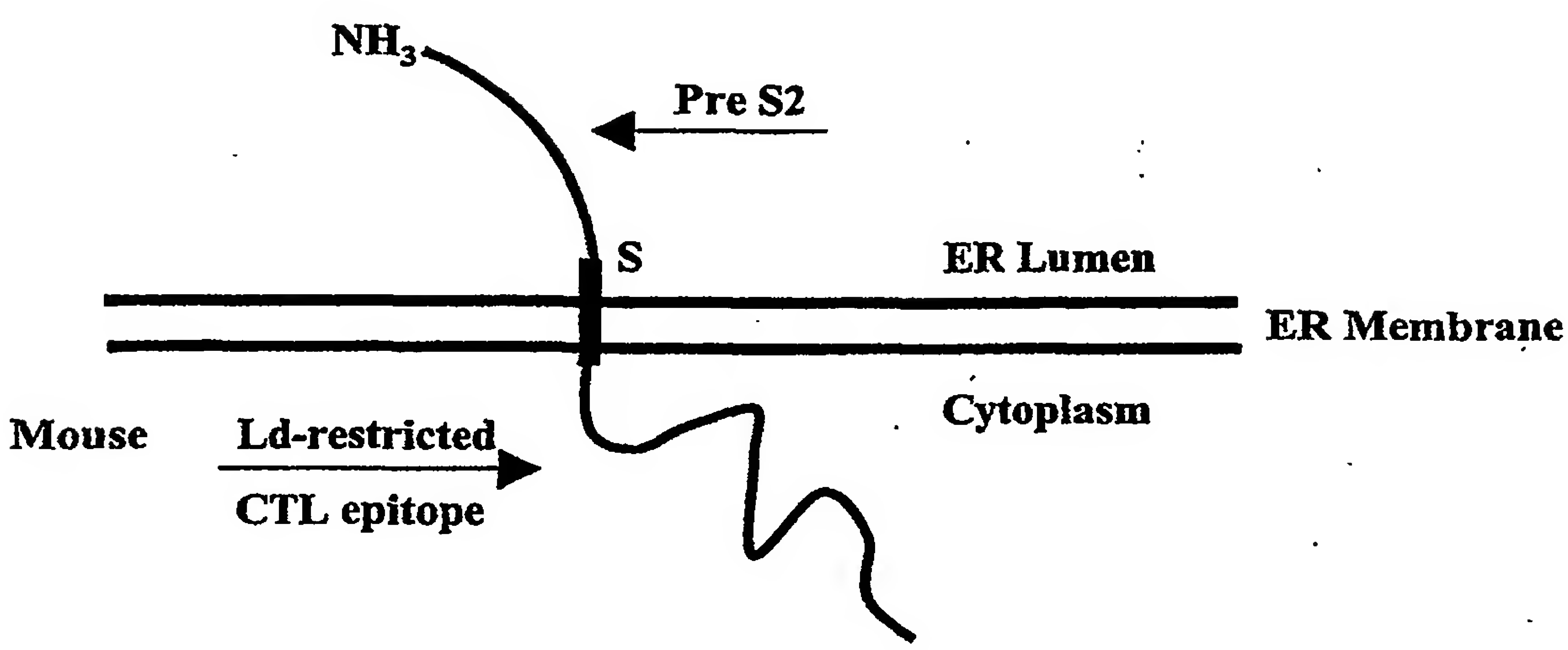


Figure 35

An example of a CTIS having heterologous epitopes attached to the cytoplasmic portion.

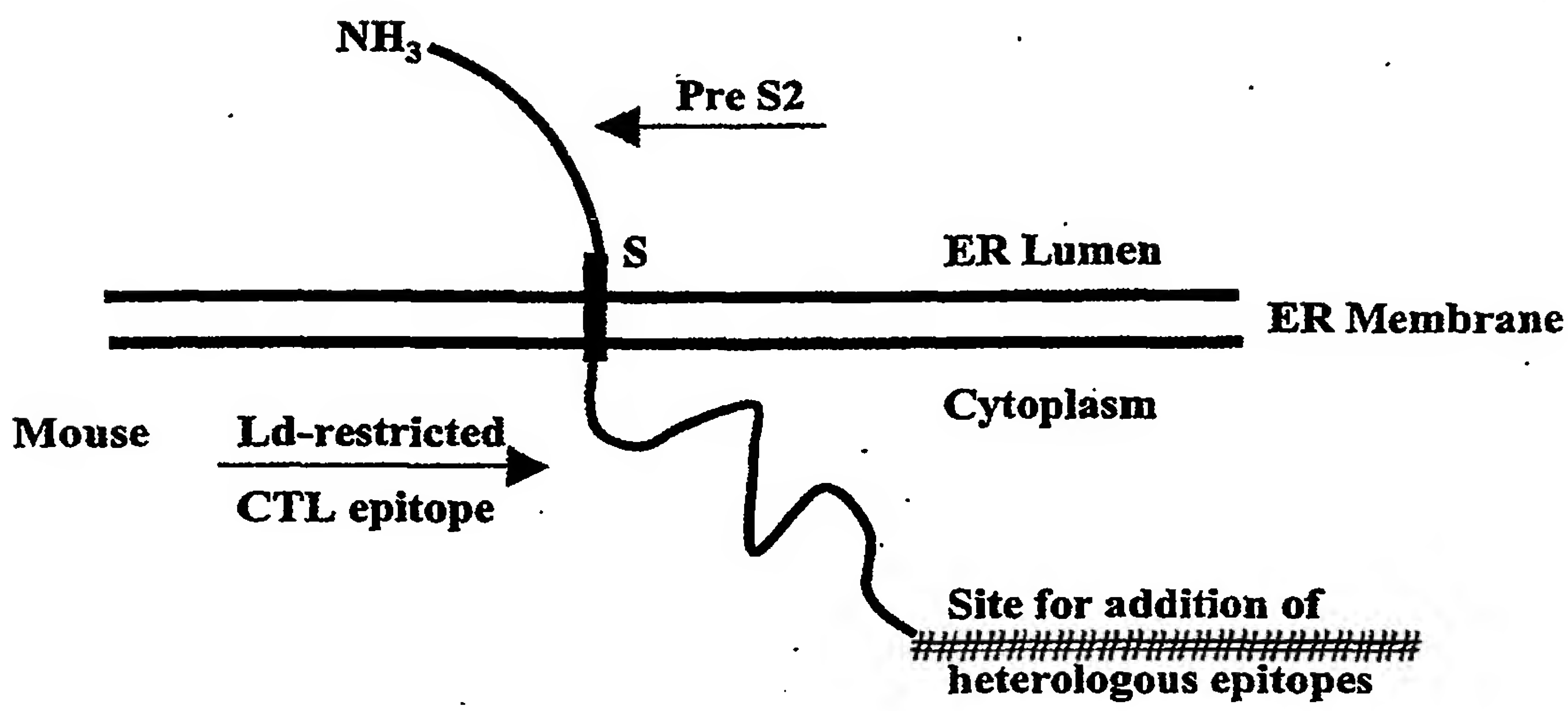


Figure 36

Method for preparing immunogenic agonist sequences (IAS).

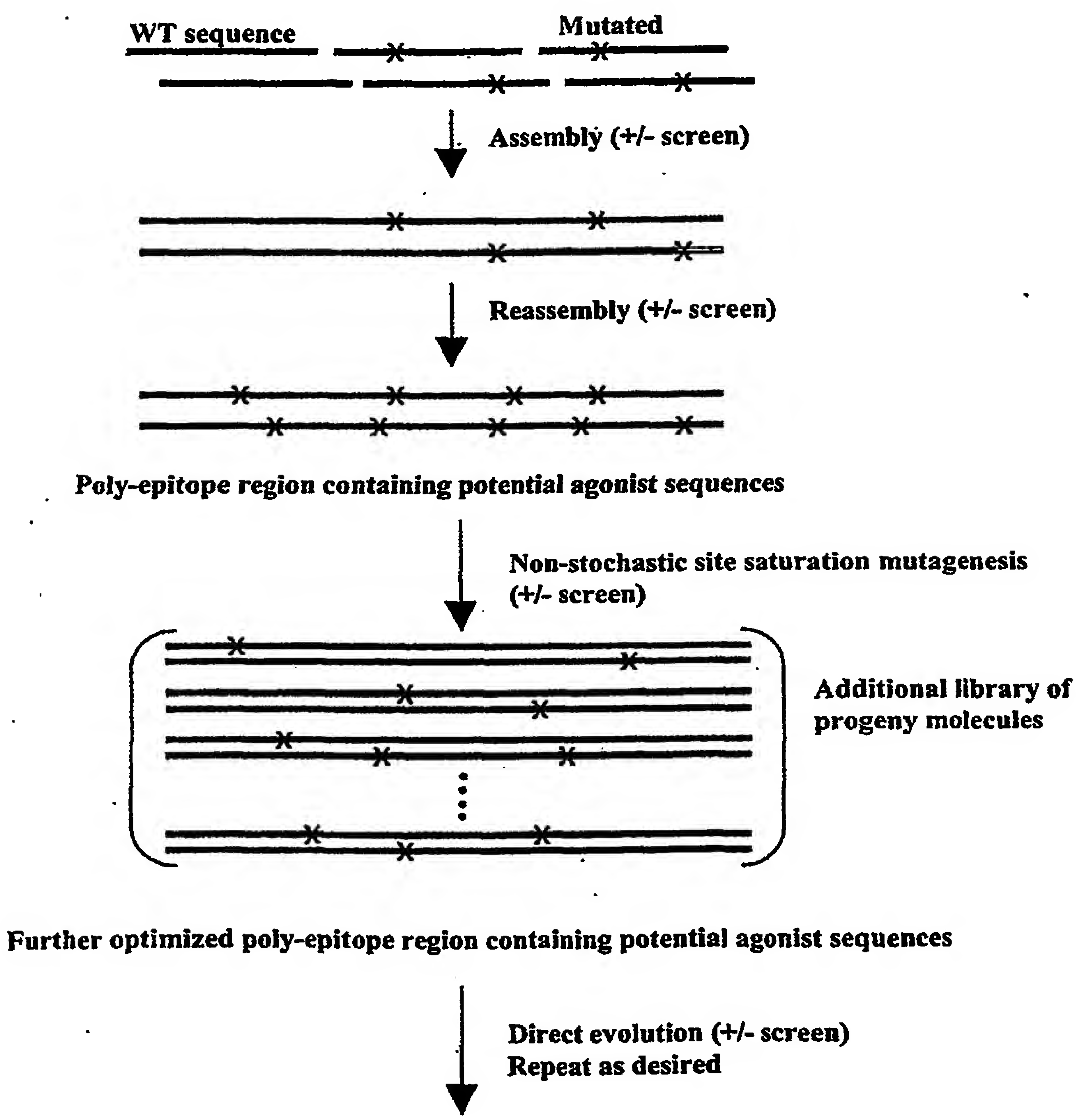


Figure 37

Improving Immunostimulatory Sequences (ISS) Using Directed Evolution.

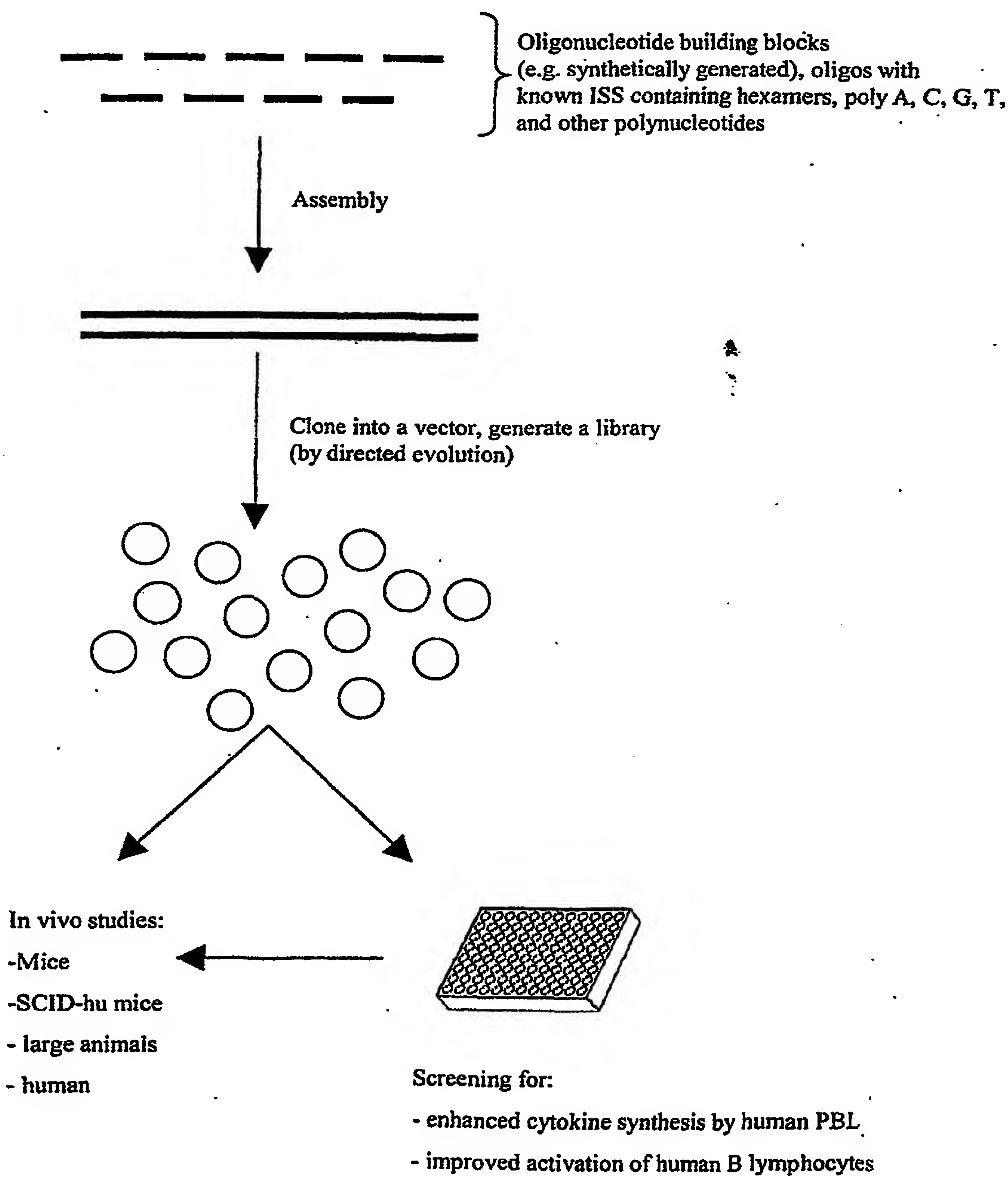


Figure 38

Screening to identify IL-12 genes that encode recombinant IL-12 having an increased ability to induce T Cell proliferation.

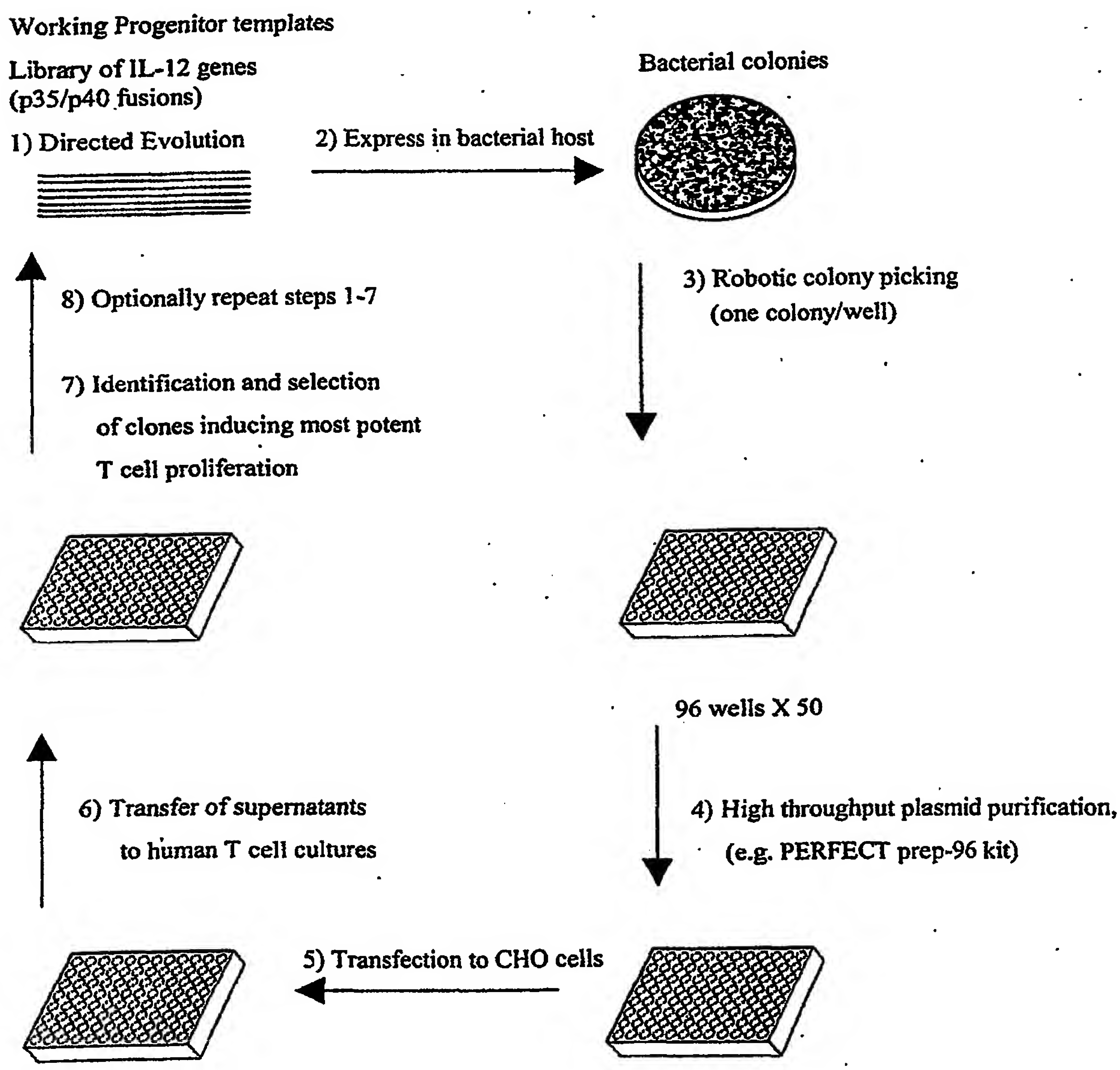


Figure 39

Model of induction of T cell activation or anergy by genetic vaccine vectors encoding different CD80 and/or CD86 variants.

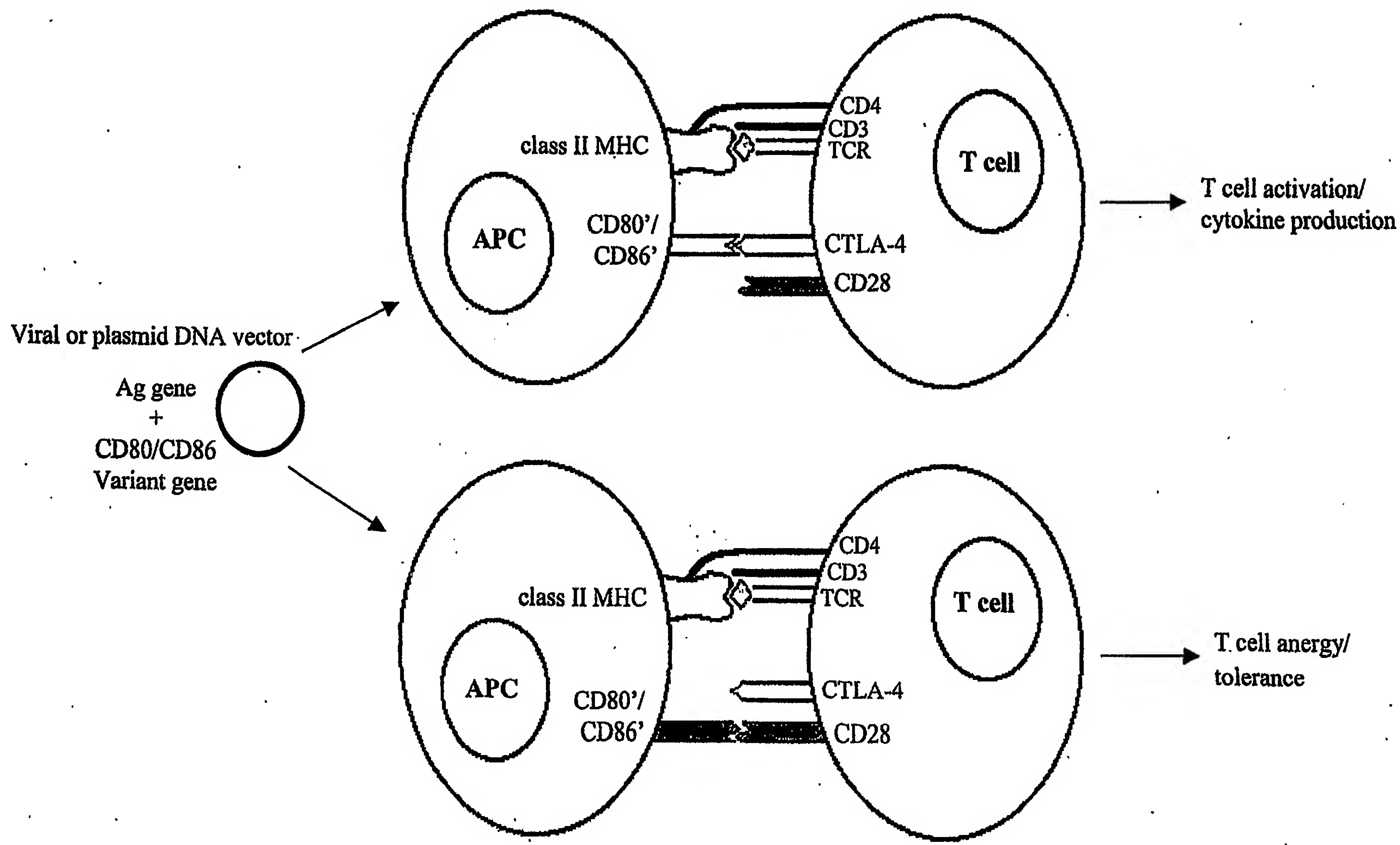


Figure 40

Screening to identify CD80/CD86 chimeric genes having an improved capacity to to induce T Cell activation or anergy.

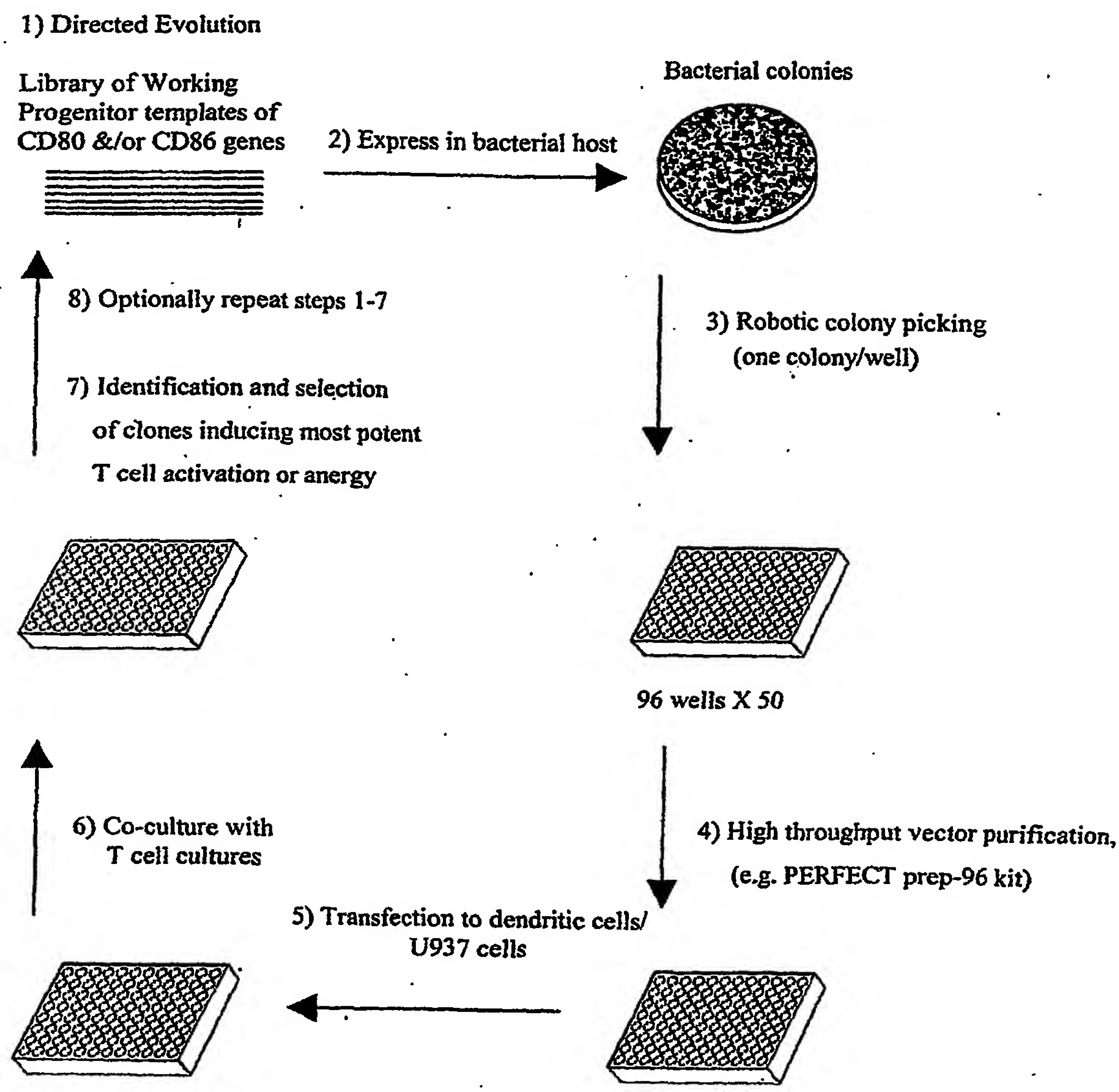


Figure 41

Figure 41. An alignment of two CMV-derived nucleotide sequences from human and primate species.

```
                       1                                                50
AF078102 Rhesus   (1)  ATCGATTTAAACTGCCCGATTGAGGTTGTGGCTGAGCAGTTTGTGAGTG-
 M67443 Towne     (1)  ---------------------------GCGATGGCATCGGACGGGGGGC

                       51                                              100
AF078102 Rhesus  (50)  -TGGTGGAAGGTAGATAGGGACACTGCGTGCCAGAAGTGCGGCTGAAGGT
 M67443 Towne    (24)  CAGGTCCGGGGCACGTGCTGGAAGCCGTGGTTAGTCGCGGGGAC-GCGGG

                       101                                             150
AF078102 Rhesus  (99)  GGGGTACATATGCGGAAGGAGGCGAGATGAGCGGGGCGAGGAGGTG-GGAGG
 M67443 Towne    (73)  GTG--------GTGCCGCA-GGAGACGCGACGGGTGCAGAGGGGTGGCGA

                       151                                             200
AF078102 Rhesus (148)  G-GGCGACGACGGG-GTGTGGAGGTTGGGGAAGCGGGTGTGCGGACGTTG
 M67443 Towne   (114)  GGTGCGCGGGAGCCAGCGCGGGCGGATGCGGGGGGGCAGGAGGCGCCCG

                       201                                             250
AF078102 Rhesus (196)  TGG-GAG--TAGGTTAGAG-GGTGTTTAGGGTGGGGACGATACGGTGGACGG
 M67443 Towne   (164)  AGGCGAGGCCAGGCCAGGCGCGGCGACAGGGAGGTGGGGTGCGGGGCAGG

                       251                                             300
AF078102 Rhesus (242)  GG-GGGTACGAGAGTATGTTAAAGAGGATGCGGTAGTGTCATTCGGGTGG
 M67443 Towne   (214)  GACGGGTACGGGCAGCGAGGTGGAGAACGGGGCGGTGAG------GGG-GG

                       301                                             350
AF078102 Rhesus (291)  GGGAGGGGGTGTAGGTGGTTTCTCGTCCTTCGGTCTGGTATAGGGAGTGGG
 M67443 Towne   (257)  AGGAGGGGACGGGGCGGA------------GG-----------GGCGGGGG

                       351                                             400
AF078102 Rhesus (341)  GAGGTGGAGAGGGGG-GGGTGGGACGAGGGT-GTTGGGTGGGAAAGAGGAA
 M67443 Towne   (285)  GAGCCGAGGGGGGAGGTGGAGGTATGTGGGCGGGCGGGCGGTCG-GGATG

                       401                                             450
AF078102 Rhesus (389)  TGGGGAGCAGTGGGGAGGATTGGGGGTGGGGTGGGTGATGGGAGT-GGTG
 M67443 Towne   (334)  CGGAGCATCGCCAGCAGCAACGTGCACGGGCGACGC-GTCGGGGGCCGGGG

                       451                                             500
AF078102 Rhesus (438)  GGGAGAGAATCGGGGAGAGTACGTGGGTGGTGCTGCGGGGGAGGTATGGA
 M67443 Towne   (383)  GGGAGCGCCGAGGCCTGCGCGTAGCGGACGCGGGGAGTC-GCGGGTCGGG

                       501                                             550
AF078102 Rhesus (488)  TTTGTGTGCGGGTATGAGTGG---GGGAGGGAGGG-GGGCTTTCAGGTAG
 M67443 Towne   (432)  CAAGCAGGTGGGGCAGGGGCGTCTCGGGGGGCTGGGGAGGGGCCTGGACGC

                       551                                             600
AF078102 Rhesus (534)  GG-GCGTCGCTGGAAGAGCTCGCGGCAGCAGGGGTGGAGG-GGAGGTGAAG
 M67443 Towne   (482)  GGCAGGGAGAACGGGTGGAA-GAGGCGGAGG-GCGAGGACAGGGCAGCGG

                       601                                             650
AF078102 Rhesus (582)  CGGGAGAGGGCCATGGGGAGGCGCGCGTCTCGGGCAGCGAGGCGCGGTGT
 M67443 Towne   (530)  TCGGGTTGG-----GGAGCAAGGAGGTGG--GAGTGGGCAGGGGGGGG

                       651                                             700
AF078102 Rhesus (632)  GAAGTGGAGGAAGTGGGGGGTGGGGAGCCGACGGAGGGAGGTGGTGTAGC
 M67443 Towne   (573)  GGCG-GACGGGCGGGGG-GG-GGGCAGGGAGAGCAGGGGGGCAACGAGGA
```

Figure 41 continued

```
                            701                                              750
AF078102 Rhesus     (682)   TAAGACCGCAGAAGAGTTTCATGGCGTTAACGTAGTAGTGAAAAGGC-GT
   M67443 Towne     (620)   T--GCAGCTGATACGTGACCAGTACCTCAAGCTGTACCTGCGAGTGCTTGT

                            751                                              800
AF078102 Rhesus     (731)   GGCCGATGGGAGGCCGATCCGTTGCGCCGTCTGG--GTACTGTGGAGAAG
   M67443 Towne     (668)   GCGAGGACGT-GCGGTCCGGCAAGCGCTTGTATCCACGTCACGCTGGGCTC

                            801                                              850
AF078102 Rhesus     (779)   CTACGTCAGATGTATGGCTTTGCTAAAATGAGAGGTGATGAGTTTAGTAA
   M67443 Towne     (717)   TGACCTGGAAGAGGACGTGACGATGACCCGCAACCGCAACGCTTCATGC

                            851                                              900
AF078102 Rhesus     (829)   TGTG--GTAGAGTATGTTAATCAAGGCATGTCTTGCGCGA------TGGTG
   M67443 Towne     (767)   GGCGCCACGAGCGCAGCGGCGTTACGGTGTTGTGTCGCAAAAATATGATG

                            901                                              950
AF078102 Rhesus     (871)   --GGTAGTTGGTGTTA-GTGCG-CGTTACGCGTAATGTTGGGAATGGTGCTG
   M67443 Towne     (817)   ATGAAAGCGGCCAAGATGTCGGAGATCATGCTGGATGTCGCCTGGTACGTC

                            951                                             1000
AF078102 Rhesus     (917)   AGCTGTCGAATAATTCTGTTGGACGAGTGCGGCGTGTGGTATGTGC-TGA
   M67443 Towne     (867)   ACACGAGCATT--GTGGCTGCT-GTGTCGCAAGAGCATCCCGGGGCTGA

                            1001                                            1050
AF078102 Rhesus     (966)   GAATGGACGAAAGCGACGTGTTT--CGTCTAGGCCGATAAGATCGAAATGT
   M67443 Towne     (914)   GCATGTCAGGTAAGCTATTGATGAAGGGGCAGCAGAT--GTTGCGTGGAGG

                            1051                                            1100
AF078102 Rhesus    (1014)   TGTTCAGATGCGGCTTTGTTAAGCTGCGTGCCATTACGCGATTTGAGCGC
   M67443 Towne     (962)   TGC----AAGCGA--TAGCGAGA-GCGTGCAACTGCGTCAGT--AG---

                            1101                                            1150
AF078102 Rhesus    (1064)   GGATTGCGTGCGGAGCGTCGTCTG----GAGAGTGAGT-GCTACTGCGTAT
   M67443 Towne    (1000)   -GATCCGGTGCGTGCGCTGTTGGTTTTTCGATATCGACTTGCGGGCTGG-AG

                            1151                                            1200
AF078102 Rhesus    (1109)   GAGGCTGGCCGTAAGCAAGATGTGTGTTGGAGGTGTATGGTGGACACAGT
   M67443 Towne    (1048)   CGCGGGCGTCAGTACAGCGAAGAGCGCACCGTGAGCA--GCGAGTATCGC

                            1201                                            1250
AF078102 Rhesus    (1159)   TAGCGCCGACGAGCTCGGTGGTGGGT-AGGAGCGCTCAGGCGGAGTGAT
   M67443 Towne    (1096)   ATGGA--GGGCAGCTTGAGTACGGACAGACGTGGG--AGCGGCACGACG

                            1251                                            1300
AF078102 Rhesus    (1208)   CGCGCTGATGATGAAATACGTAGCAGTGGGTGTTGGGGACGTAGTGATTT
   M67443 Towne    (1142)   AGGGTGGCGCCCAGGGCGAGGAGGACGGCTGGACCAGCGGATCGGACGCC

                            1301                                            1350
AF078102 Rhesus    (1258)   GATGCCTGCGGGAGGTATTGCAACAGAATCAGAATTGGTCGTGGCGGTGGA
   M67443 Towne    (1192)   GACGAGGAAGTC-GTTACCACCGAGCGCAAGACGCCCCGCGTTAGCGGCG

                            1351                                            1400
AF078102 Rhesus    (1308)   GGAGTGTCGCGGTTCTAGAAGGCGG-GCGAAGGGGTGCGTGCGGAG--GAG
   M67443 Towne    (1241)   GCGGCGCCGATGG-----GGGGCGGCTGGACTTGCGCGGGCCGGGAACGCG
```

Figure 41 continued

```
                              1401                                              1450
AF078102 Rhesus  (1355) AATGATGAGTTAAGGCAGAAAACGTCTGATGGTACTGTGATGTTTGGTGT
  M67443 Towne   (1286) AATCAGCA-TCCTGGGCCACGGGCTGCACGGCGGGCGTTATGACAGGCGG

                              1451                                              1500
AF078102 Rhesus  (1405) A-GACAGAGGTTTTTCTGGCAGAAGCGTAAAGTTGGAGGGTGAAAGGCGT
  M67443 Towne   (1335) CCGCGTTAAGGCCGAGTGG----AGCGTCGCGCCGGAAGAGGACACCGGC

                              1501                                              1550
AF078102 Rhesus  (1454) GACTACAGAGAAGAGGAGGAAGATGATGATAACCAGTCAGAAGAAACGCC
  M67443 Towne   (1381) GAGGATTGCGACAACGA--AATCC----ACAATCGGGCGTGTTCACGTG

                              1551                                              1600
AF078102 Rhesus  (1504) TCAGGATCAGGCTGTTGTCATTGTTTCAGATGAAACAAACGGCAGTGTTG
  M67443 Towne   (1425) GGCGGCCTGGCAGGCCGGCATCCTGGGCAGCAACCTGGTGCCCATGGTTG

                              1601                                              1650
AF078102 Rhesus  (1554) ATTGTTATGATGATGAGAGTGAGTCAT-GTCTG--TCGT-CACATAGCGA
  M67443 Towne   (1475) CTACGGTTCAGGGTCAGAATCTGAAGTAGCAGGAGTTCGTTGTGGAGGCC

                              1651                                              1700
AF078102 Rhesus  (1600) TGATGCAGCAGCCGGAGACATTATGGCGATAATCCAAAAGCAATCGCA--
  M67443 Towne   (1525) --AAGGAGATGTACCG--CATCTTCGCCGAATTGGAAGGCGTATCGCAGC

                              1701                                              1750
AF078102 Rhesus  (1648) ----------AAACGAAGAGCGGGTTAGCAATGAAAGAGAATTGGTTAGTGC
  M67443 Towne   (1571) CCGCTGCGCAACGCAAAACGTGGCCGCGACCGGCAACACGCCT-TGCGCGG

                              1751                                              1800
AF078102 Rhesus  (1689) GGGGTGCA---GGAGGA-GGTAAACATATGAAAAAGTGGCATTGTTGCGT
  M67443 Towne   (1620) GCGATGCATCGGCTGGACGGCCAAAAAGCACCGAGGTTGAGCCACCGGGC

                              1801                                              1850
AF078102 Rhesus  (1735) GGAAAGGGTTAGCA------TTTATGACGCGTTGAATGCTAATATTGAGT
  M67443 Towne   (1670) GCGCACGCTTAGGACGACTCTATAAAAACGCACGTCCAGTCAGACAGGGG

                              1851                                              1900
AF078102 Rhesus  (1779) GCTTTGATTAAACAATGCATTTGTATTGAAATATCTAATTCGGGT--GTA
  M67443 Towne   (1720) AGTTTTGGCCGCCA---CACCTGTCGCGGCTGCTATATTTGGCACAGTTG

                              1901                                              1950
AF078102 Rhesus  (1827) CGGTGTGATTTCGTGCATGGAGTGGTGGGAAAGAGATTAGGTATTTAGAGA
  M67443 Towne   (1767) CGGGAAGCCTTCCGGA--GCTCCCACGAAGAACGGTT-GACCTTGGCGGA

                              1951                                              2000
AF078102 Rhesus  (1877) -GACGTATGAGTTGTGAAAGCTGAGTGAAGATCATGCAATTGTTGGAGGA
  M67443 Towne   (1814) TGCGCTGAGCGCCCCC----GTCATCGCGGCTTCGCGATGTCTCAGGCAT

                              2001                                              2050
AF078102 Rhesus  (1926) GGTCGGGGTGGTTCGTTCTGTGTTTGT-GTTACGAGTTTAGATTTTATTA
  M67443 Towne   (1860) CGTCG---TCGCCCGGTGAGGGACCGTCGTCGGAAGCGGCGGCGATCAGC

                              2051                     2077
AF078102 Rhesus  (1975) TGGTTGAAAGTTTTGTATTTCTAA---
  M67443 Towne   (1907) GAGGCGGAAGCGGCG-AGCGGAAGCTT
```